1934

Sugar Cane Variety Report 1933

Gouaux B. C

Louisiana Bulletin No. 162. March, 1918.

Agricultural Experiment Station

of the

Louisiana State University and
A. & M. College

Baton Rouge

The Deterioration of Cane Sugars in Storage; Its Causes and Suggested Measures for Its Control.

BY

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Baton Rouge, La.
Ramires-Jones Printing Co.
1918

The investigation of sugar deterioration, of which the present publication is a complete report, has occupied a period of approximately nine years. While other reports have been published during that time, they were intended either as preliminary reports of progress, as in Bulletin 125, or as reports of collateral investigations, as in Bulletins 146 and 153. Shortly after embarking upon the main course of the investigation the writer recognized the necessity of building a broader foundation for the work by conducting several subordinate lines of research. It was mainly through these that the ideas discussed in the present publication were obtained.

In taking a retrospective view of the course over which the investigation has led, the author feels that whatever meagre success has crowned his efforts, it is, in a large measure, due to the unstinted assistance of those with whom it has been his great pleasure to be associated. The author feels constrained to admit that the success of the present investigation does not fulfill the expectations which might have been entertained for it, in view of the almost ideal conditions for research with which he has been surrounded. Whatever success has attended the author's efforts is due to the freedom which he has been allowed in following out his own ideas; to the opportunities which have been given him to take advanced training in a foreign country, and to the kind interest his associates have taken in his work and their untiring efforts in its behalf.

The writer desires to express his sincere thanks to Dr. C. A. Browne, of the N. Y. Sugar Trade Laboratory, for looking over a part of his manuscript and for many valuable suggestions given through correspondence. Also to Dr. Charles Thom and Miss Margaret Church, of the Bureau of Chemistry, for identifying the mould cultures obtained from sugars. The writer's thanks are also due to Dr. C. E. A. Winslow, of the New York Museum of Natural History, for pure cultures of the potato group of bacteria used in one of the experiments. For the kindly interest taken in his problems and for the many valuable suggestions given in connection therewith, the writer acknowledges his sincere appreciation to Prof. Alfred Koch, of the Landwirtschaftliche Institut of Goettingen, in whose laboratory two semesters

were spent upon this investigation. To Mr. Inami, Mr. Hargrove and Mr. Sullivant, who respectively filled the position of assistant at various times, the author acknowledges his sincere indebtedness. Also to Assistant Director Taggart, and Dr. Max A. Schneller, research chemist, the author is indebted for very helpful suggestions and constant offers of assistance.

The investigation has been greatly facilitated at all times by the willingness of those engaged in the sugar industry in this locality to furnish samples of various materials. The writer wishes to express his thanks for such coöperation on the part of the managements of most of the sugar factories throughout the State. Also to Mr. J. M. McFetridge, superintendent of the American Sugar Refinery at Chalmette, who donated several lots of coarse granulated sugar, the writer desires to express his thanks.

Owing to the large volume of experimental data obtained in the extensive course of this investigation, it was found to be impracticable to include all of it in this report. In order to keep the volume of the report within an appropriate size, it was necessary to restrict the publication of experimental results to those considered essential to the successful demonstration of the theories which the experiments were intended to illustrate. In making this selection a careful average was taken of the results from duplicate experiments, so that the published results may be regarded as entirely representative of all of the available experimental data on the subject.

This Bulletin was submitted for publication August, 1917

PART I.

OBSERVATIONS UPON THE CHANGES IN THE CHEMICAL COMPOSITION OF SUGAR SAMPLES IN STORAGE.

The observation of the changes in the chemical composition of sugar samples during storage constitutes an indispensable means of obtaining the necessary preliminary data for the basis of an investigation of the causes of sugar deterioration and the influences predisposing thereto. It is by this means that we are enabled to study the relative tendency of various types of sugar either to successfully resist or to yield to the activities of those agencies which are capable under certain conditions of inducing their deterioration. It is only by such observations upon sugars in their natural condition that we can feel certain of obtaining results that are commensurate with the actions of the natural micro-flora complexes upon sugars unchanged in composition by efforts to sterilize them prior to inoculation with pure cultures. The purpose of this part of the investigation, however, was not only to observe the behavior of different types of sugar in storage, with a view of correlating as far as possible the various factors influencing it, but also to make a more extended investigation of the species occurring in sugars of various types and from various sources. It was anticipated that the last-named effort might result in the isolation from sugars of species of micro-organisms hitherto overlooked, whose demonstrated presence therein might serve to explain many apparently inconsistent results that have been previously obtained in the observation of the changes in the chemical composition of sugars in storage.

For these experiments a number of sugar samples were collected from the various plantations throughout the State during the grinding season of 1914. These samples were collected in special glass jars sent from this laboratory to the various sugar houses. The samples were thoroughly mixed promptly after their arrival at the station, then analyzed and divided into two portions, one of which was transferred to large Erlenmeyer flasks and placed in an incubator at 34°C. The other portion of the sample

was kept in a tightly closed fruit jar at room temperature. There were two principal factors that from the beginning of the experiment were chosen for special study. These factors are the solid non sucrose-moisture ratio of sugars and the number of micro-organisms they contain. The solid non sucrose-moisture ratio is believed to be a reliable criterion of the keeping quality of raw sugars. It has been used as the basis for the "factor of safety"[1] established by the Colonial Sugar Company of Australia, which states that the water in a raw sugar should not be more than half the non sucrose, or when the percentage of water in a sugar divided by 100 minus the polarization, is less than 0.333, the sugar will not deteriorate. The other predisposing factor in sugar deterioration would naturally be supposed to be the degree of infection of sugars with micro-organisms. If the causative agency in sugar deterioration is biological in nature, then the number of micro-organisms in sugar should vary directly with the rapidity of the rate of their deterioration.

[1]Cane Sugar, Noel Deerr, p. 383.

TABLE I.

Table Showing Changes in Composition of Sugar Samples in Storage. Samples Having a Solid Nonsucrose-Moisture Ratio of (.100-.200).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS		INCUBATOR DRY BASIS			Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
23	La. Sec.	1/12/15	88.53	90.25	3.83	2.18	.188	90.46	92.18	90.46	92.18	3.83	2.18	.188	120,000
"	"	2/20/15	87.74	90.59	3.34	2.20	.171	89.67	92.52	90.10	92.94	3.46	2.89	.232	
"	"	6/1/15	87.84	90.02		2.38	.195	89.94	92.12	91.79	92.89	2.31	4.03	.204	
"	"	8/7/15	87.34	89.84	3.84	2.15	.169	89.22	91.72	—	—	—	—	—	
32	La. 96 T.	1/14/15	95.89	97.38	1.37	0.72	.175	96.59	98.08	96.59	98.08	1.37	0.72	.175	8,000
"	"	2/25/15	95.95	97.24	1.56	1.10	.271	96.96	98.25	97.79	98.57	1.45	1.84	.462	
"	"	6/18/15	96.10	97.39	1.25	1.08		97.14	98.63	98.11	99.16	0.45	1.24	.398	
"	"	8/19/15	94.76	97.02	1.42	0.83	.158	96.50	98.76	—	—	—	—	—	
41	La. Y. C.	12/15/14	99.60	99.82	0.13	0.06	.150	99.66	99.88	99.66	99.88	0.13	0.06	.150	6,000
"	"	3/2/15	99.23	99.87	0.13	0.06		99.36	100.00	99.26	99.92	0.15	0.06		
"	"	6/17/15	99.60	99.90	0.10	0.10		99.70	100.00	99.46	100.00	0.13	0.16		
"	"	8/24/15	99.13	99.90	0.10	0.03		99.23	100.00	—	—	—	—	—	—

TABLE II.

Table Showing Changes in Composition of Sugar Samples in Storage.
Samples Having a Solid Nonsucrose-Moisture Ratio of (.200-.300 incl.).

Sample No.	Source and Type	ROOM TEMPERATURE Date	S. P.	S. C.	R. S.	Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS S. P.	S. C.	S. P.	INCUBATOR DRY BASIS S. C.	R. S.	Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
8	Cuban 96	9/23/14	96.94	97.90	1.13	0.80	.261	97.72	98.68	97.72	98.68	1.13	0.80	.261	1,300
"	"	10/23/14	96.75	97.23	1.13	0.77	.236	97.50	97.98	98.43	98.97	0.53	1.66	.522	"
9	Cuban 96	9/23/14	95.35	96.50	2.10	1.26	.270	96.56	97.71	96.56	97.71	2.10	1.26	.270	2,000
"	"	10/23/14	95.12	95.67	2.30	0.89	.182	95.97	96.52	96.57		2.54	2.71	.453	"
10	Cuban 96	9/23/14	94.80	95.96	2.52	1.33	.255	96.07	97.23	96.07	97.23	2.52	1.33	.255	4,800
"	"	10/23/14	94.16	94.89	2.63	1.08	.184	95.18	95.91	93.80	94.27	4.45	2.67	.308	"
11	Cuban 96	9/23/14	94.75	95.93	2.60	1.26	.240	95.95	97.13	95.95	97.13	2.60	1.26	.240	2,900
"	"	10/23/14	94.54	95.73	2.86	1.04	.190	95.53	96.72	93.68	94.60	4.09	2.33	.275	"
12	Cuban 96 A	10/9/14	95.36	96.30	1.90	1.15	.247	96.46	97.40	96.46	97.40	1.90	1.15	.247	43,300
"	"	1/6/15	95.21	96.26	1.30	1.41	.294	96.56	97.61	94.73		2.85	4.68	.492	"
13	Cuban 96 B	10/9/14	95.28	96.08	2.05	1.06	.224	96.30	97.10	96.30	97.10	2.05	1.06	.224	26,000
"	"	1/6/15	95.20	95.68	2.00	1.17	.243	96.32	96.80	—	—	—	—	—	—
14	Cuban 96 C	10/9/14	95.70	96.48	1.22	0.97	.225	96.63	97.41	96.63	97.41	1.22	0.97	.225	23,300
"	"	1/6/15	95.41	95.73	1.10	1.38	.300	96.73	97.05	96.58	96.81	0.68	.278	.460	"
15	Cuban 96 T	10/9/14	93.67	94.63	2.77	1.59	.248	95.15	96.11	95.15	96.11	2.77	1.59	.248	66,600
"	"	1/6/15	93.42	94.50	2.30	1.85	.281	95.15	96.23	—	—	—	—	—	—
30	La. 96 T	1/14/15	95.99	97.47	1.64	0.97	.242	96.93	98.41	96.93	98.41	1.64	0.97	.242	10,000
"	"	2/23/15	95.80	96.50	2.18	1.01	.244	96.77	97.47	97.70	97.80	1.72	1.95	.467	
"	"	6/5/15	95.91	97.78	1.73	1.01	.246	96.88	98.75	98.22	99.45	0.35	1.75	.504	
"	"	8/19/15	95.72	98.07	1.70	0.94	.219	96.62	98.97	—	—	—	—	—	
38	P. R. 96 T	1/18/15	95.12	95.70	2.79	1.36	.278	96.42	97.00	96.42	97.00	2.79	1.36	.278	30,000
"	"	2/27/15	94.71	95.45	2.86	1.19	.224	95.84	96.58	96.65	96.21	3.18	2.33	.419	
"	"	6/15/15	94.01	95.55	2.63	1.28	.213	95.22	96.76	94.98	94.16	3.92	3.35	.413	
"	"	8/21/15	94.41	95.97	2.40	1.30	.232	95.64	97.20	—	—	—	—	—	

TABLE II.---Continued.

Table Showing Changes in Composition of Sugar Samples in Storage.
Samples Having a Solid Nonsucrose-Moisture Ratio of (.200-.300).

Sample No.	Source and Type	ROOM TEMPERATURE Date	S. P.	S. C.	R. S.	Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS S. P.	S. C.	INCUBATOR DRY BASIS S. P.	S. C.	R. S.	Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
28	La. 96 T.	1/14/15	96.80	97.07	1.02	0.94	.294	97.71	98.98	97.71	98.98	1.02	0.94	.294	8,000
"	"	2/20/15	97.00	97.34	1.40	0.96	.320	97.94	98.24	98.11	98.42	1.28	1.14	.380	
"	"	6/1/15	97.20	97.53	1.25	0.83	.296	98.01	98.34	98.75	98.72	0.71	2.18	.502	
"	"	8/17/15	96.90		1.03	0.71	.229	97.59	—	—	—	—	—	—	
34	La. 96 T.	1/14/15	93.78	96.04	1.13	1.89	.303	95.56	97.82	95.56	97.82	1.13	1.89	.303	8,000
"	"	2/25/15	93.13	94.68	2.60	2.09	.304	95.08	96.63	96.10	97.55	2.52	2.40	.389	
"	"	6/8/15	94.00	95.63	1.67	1.55	.258	95.48	97.11	94.28	96.24	3.97	3.38	.383	
43	La. 88°	1/18/15	86.04	87.66	5.63	4.29	.307	89.74	91.39	89.74	91.39	5.63	4.29	.307	12,000
"	"	3/4/15	85.95	87.44	5.36	4.18	.297	89.54	91.04			3.84	4.78	.441	"
"	"	6/17/15	87.97	88.78	4.36	4.42	.367	91.86	92.67	92.95	93.40	1.87	3.72	.358	
"	"	8/24/15	87.82	89.20	4.80	3.94	.323	91.29	92.67	—	—	—	—	—	
39	P. R. (J)	1/18/15	95.11	95.23	2.54	1.55	.316	96.59	96.71	96.59	96.71	2.54	1.55	.316	44,000
"	"	3/2/15	95.02	95.74	2.16	1.71	.343	96.65	97.37	97.45	97.16	1.63	2.73	.531	"
"	"	6/15/15	94.32	96.07	2.13	1.45	.255	95.69	97.44	96.51	96.66	2.32	2.50	.427	
"	"	8/21/15	94.81	95.96	1.63	1.50	.289	96.24	97.39	—	—	—	—	—	

TABLE III.

Table Showing Changes in Composition of Sugar Samples in Storage.
Samples Having a Solid Nonsucrose-Moisture Ratio of (.300-.400 incl.)

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS			INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
46	La. 96 T.	1/18/15	96.20	96.60	1.66	1.31	.344	97.47	97.87	97.47	97.87	1.66	1.31	.344	6,000
"	"	3/4/15	95.79	96.63	1.65	0.95	.344	96.71	97.55	96.48	97.23	1.76	1.33	.277	"
"	"	6/19/15	95.20	96.98	1.34	0.90	.187	96.06	97.84	98.47		0.08	1.29	.462	"
44	La. P. G.	1/18/15	99.50	99.42	0.06	0.17	.340	99.67	99.59	99.67	99.59	0.06	0.17	.340	7,000
"	"	3/4/15	99.40	99.79	0.08	0.03	.050	99.43	99.82	98.88	99.68	0.67	0.37	.248	"
"	"	6/19/15	99.10	99.94	0.13	0.06	.066	99.16	100.00	95.67	97.82	2.83	1.33	.237	"
"	"	8/24/15	99.00	99.54	0.08	0.06	.060	99.45	100.00	—	—	—	—	—	
37	P. R. (G)	1/18/15	95.81	96.39	1.95	1.43	.341	97.19	97.77	97.19	97.77	1.95	1.43	.341	1,460
"	"	2/27/15	95.79	96.41	2.76	0.93	.220	96.69	97.31	97.48	97.10	2.43	2.05	.457	"
"	"	6/12/15	94.99	95.28	1.64	0.96	.191	95.91	96.20	96.74	—	1.54	1.70	.347	"
"	"	8/21/15	95.41	96.94	1.75	1.12	.241	96.48	98.01	—	—	—	—	—	
45	La. 96 T.	1/18/15	95.90	96.14	1.47	1.40	.341	97.25	97.49	97.25	97.49	1.47	1.40	.341	1,000
"	"	3/4/15	95.50	96.25	1.53	1.09	.242	96.55	97.30	96.88	97.53	1.56	1.33	.302	"
"	"	6/19/15	95.30	97.15	1.40	1.03	.219	96.29	98.14	96.72	98.15	0.08	0.34	.094	"
"	"	8/24/15	96.10	97.28	0.21	1.21	.310	97.27	98.45	—	—	—	—	—	
29	La. 96 T.	1/14/15	94.63	95.97	1.65	2.00	.372	96.53	97.87	96.53	97.87	1.65	2.00	.372	633,000
"	"	2/23/15	95.33	95.23	1.64	2.00	.428	97.24	97.14	96.76	96.99	1.06	2.54	.450	"
"	"	6/5/15	94.16	—	1.43	2.74	.469	96.75	—	91.97	93.79	5.18	4.46	.372	"

TABLE IV.

Table Showing Changes in Composition of Sugar Samples in Storage.
Samples Having a Solid Nonsucrose-Moisture Ratio of (.400-.500).

Sample No.	Source and Type	ROOM TEMPERATURE					Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS		INCUBATOR DRY BASIS			Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.				S. P.	S. C.	S. P.	S. C.	R. S.			
25	La. 2nds	1/12/15	89.18	90.28	2.40		4.45	.411	93.25	94.35	93.25	94.35	2.40	4.45	.411	
"	"	2/20/15	90.10	92.27	1.16		4.83	.487	94.46	96.63	94.13	95.42	0.37	5.45	.506	323,700
"	"	6/1/15	89.78	91.23	0.98		4.45	.435	93.88	95.33	85.90	87.48	7.69	6.76	.344	
"	"	8/17/15	89.30	90.73	1.26		4.78	.446	93.57	95.00	—	—	—	—	—	
35	La. Y. C.	1/18/15	99.06	99.56	0.52		0.44	.468	99.50	100.00	99.50	100.00	0.52	0.44	.468	236,700
"	"	2/27/15	98.92	99.11	0.48		0.33	.305	99.40	99.59	97.76	98.40	1.87	1.19	.321	
"	"	6/12/15	98.89	99.43	0.77		0.26	.234	99.15	99.69	94.14	95.26	4.90	2.49	.304	
"	"	8/21/15	98.30	—	1.03		0.34	.200	98.64	—	—	—	—	—	—	
31	La. Y. C.	1,14/15	98.79	99.30	0.19		0.70	.578	99.49	100.00	99.49	100.00	0.19	0.70	.578	660,000
"	"	2/25/15	98.49	—	0.55		0.66	.437	99.15	100.00	98.34	—	1.45	1.06	.392	
"	"	6/5/15	97.29	98.68	1.26		0.66	.317	97.94	99.33	96.39	98.29	2.77	1.65	.318	
"	"	8/19/15	97.19	98.98	1.75		0.59	.209	97.57	99.36	—	—	—	—	—	

TABLE V.

Table Showing Changes in Composition of Sugar Samples in Storage. Samples Having a Solid Nonsucrose-Moisture Ratio of (.500).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS		INCUBATOR DRY BASIS			Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
36	La. 96 T.	1/18/15	97.32	98.37	1.19	1.60	.596	98.88	99.83	98.88	99.83	1.19	1.60	.596	342,143
"	"	2/27/15	97.01	97.32	0.97	1.39	.464	98.36	98.67	97.23	97.58	1.94	2.41	.476	"
"	"	6/12/15	94.62	95.70	2.08	1.84	.342	96.37	97.45	92.66	92.40	5.78	4.39	.390	"
"	"	8/21/15	93.33	95.60	2.70	1.88	.280	95.09	97.37	—	—	—	—	—	—
26	Undried Gran.	1/12/15	97.94	97.94	0.19	2.10	1.019	100.00	100.00	100.00	100.00	0.19	2.10	1.019	741,260
"	"	2/20/15	97.62	98.32	0.037	1.72	.722	99.30	100.00	99.36	100.0	0.37	1.57	.720	"
"	"	6/1/15	99.29	99.29	0.29	0.71	1000	100.00	100.00	98.18	98.83	1.60	2.10	.546	"
"	"	8/17/15	99.09	99.79	0.51	0.21	.230	99.30	100.00	—	—	—	—	—	—

DISCUSSION OF RESULTS.

A critical examination of the preceding tables shows that a solid non sucrose-moisture ratio of 3-1 does not invariably prevent a sugar from deteriorating. Exceptions are also to be observed in the case of the non deterioration of samples in which the percentage of solids non sucrose was less than three times the percentage of moisture. But with both classes of exceptions, the inadequacy of this criterion is susceptible of explanation. On the one hand, the first noted discrepancy is most often observed in cases of white sugar, both plantation granulated and yellow clarified, and often also with sugars which have been washed up to 96 test. In such cases there is a tendency, no doubt, for the films surrounding the sugar crystals to be of a lower density than one might expect from the same ratio of solid non sucrose to moisture in a natural molasses. We find, in other words, that the validity of this factor decreases in proportion as we deviate from a film composed of a natural molasses, and begin to deal with washed sugars where the molasses films are artificially diluted. And this is what we have in washed sugar, whether it be 96 test or a white plantation sugar. In the proportion that the sugar is increased in polarization test by washing, just so much is its purity raised, and hence less solids non sucrose left in the film. Hence a lower density film is the result, and a greater tendency towards deterioration. Just the reverse is the tendency to deterioration of low grade 96 test sugar, and particularly of seconds. Here we have a film of low purity molasses, which tends to show a solid non sucrose-moisture ratio indicative of a lower density film than probably exists. In these cases it will be noted that many samples did not deteriorate, when the solid non sucrose ratio would have led one to expect a rapid destruction of sucrose in storage. In commenting upon this same question of the validity of the solid non sucrose-moisture ratio as a criterion of the keeping quality of sugars, Browne,[1] who has made a most comprehensive study of the deterioration of sugar in storage, writes as follows:

[1]La. Planter, Vol. LIV, No. 18, 1897, p. 28.

"If we suppose no dissolved sucrose to be present the limiting ratio of $\frac{W}{100\text{-}Sucrose} = 0.25$ *is represented by a film consisting of 25% water and 75% non sucrose, and this it will be noted corresponds approximately to the percentage of water and solids in low grade molasses. The saturation of the water in low grade Philippine sugars with non sucrose ingredients would thus be the explanation of why these sugars do not deteriorate. The superior keeping quality of many molasses sugars and the susceptibility of the higher grades of soft refinery sugars to deteriorate are also thus explained."*

* * * * *"According to this viewpoint, it is wrong in principle to bring low grade sugars intended for storage, to a high degree of purity by washing, and doubly wrong when the wash water employed for this purpose is taken, as is often done, from the cooling tower or from other infected sources."* A comparison of the deterioration of the room temperature and incubator stored samples showed few cases where any acceleration of deterioration could be attributed to the higher temperature of the incubator. In most cases the greater deterioration could be directly traced to the increase in moisture absorbed in the more humid atmosphere of the incubator. We come next to an extremely interesting and significant fact in connection with the changes in the composition of sugars in storage. The significant fact to which we shall now take occasion to refer, is the successive increase and decrease in reducing sugars of the same sample at different periods of storage. It will be noted that these successive changes in reducing sugars follow a corresponding change in the moisture content, and also in the solid non sucrose-moisture ratio. Let us take, for example, Sample No. 34. Here we have a reducing sugar increase from 1.13 per cent to 2.60 per cent, while the per cent moisture was increasing from 1.89 to 2.09. However, during the next period of storage the moisture decreased to 1.59 per cent and the reducing sugar also decreased to 1.67. In the latter period there was a solid non sucrose ratio of .258 as compared with .303 during the first period. A similar change is observed in the case of Sample No. 37. The fact that sugars may decrease in reducing sugar and increase in polariza-

tion during storage has often been noted. It is most frequently observed in the case of low grade sugars having a high percentage of reducing sugar. Noel Deerr and Norris[1] called attention to this behavior of sugars in storage in their investigations of sugar deterioration. They also refer to a similar observation of Watts[2] and Tempany[3]. This phenomenon was further commented on by Owen[4] in 1916 in a paper presented before the Louisiana Planters' Association. As an illustration of this decrease in reducing sugar in a sample during storage, No. 43 may be taken as a typical case. Here we have a second sugar, with a solid non sucrose-moisture ratio of .307. The incubator sample of this sugar decreased in reducing sugar from 5.63 to 1.67 and increased in sucrose Clerget on the dry basis from 91.39 to 93.40. The writer has found that this behavior of sugar in storage seems to be definitely associated with a low factor of safety. In fact, it is the rule rather than the exception for the changes in the composition of raw sugars with a factor of less than .300 to be confined to the destruction of reducing sugar. As we have already noted, a lower factor has to be conformed to by white sugar and washed 96 test and a higher can be maintained in the case of seconds without danger of deterioration. Regarding the causes of this loss in reducing sugars during the storage of sugars, we will resume its discussion in Part II of this investigation. Suffice it here to say that this fermentation phenomenon is due to the suppression of the sucrose destroying power of certain groups of micro-organisms by the density of the film of molasses around the sugar crystals. Under this condition of supra maxima densities for the inversion of sucrose, the destruction of reducing sugar can still proceed. Hence the phenomenon can be taken as a definite indication of a density of molasses film around the sugar crystal, beyond that in which invertase can function.

As has already been stated, the sugar samples that were kept at room temperature were in jars provided with closely fitting metal covers. It must not be supposed, therefore, that the behavior of these samples in storage is entirely comparable with

[1]The deterioration of sugar in storage. Bul. 24, Hawaiian sugar planter.
[2]Agricultural News—Vol. IV, S. 98.
[3]West Indian Bulletin, Vol. VII, No. 3.
[4]Some observations on the deterioration of sugar in storage.

that of the original sugar when stored in bags or in barrels in warehouses.

Browne[1] has pointed out that deterioration frequently occurs in sugar samples when no evidence of deterioration can be detected in the stored sugar from which the sample was drawn. The reverse case is also often observed where, owing to bad warehousing, the sugars stored in bags become moist and deteriorate, while the samples being kept in stoppered bottles retain their original composition. In brief, it may be assumed that any difference in behavior of the sample and the original sugar stored in a warehouse would likely be due to the fact that the latter, being in more direct contact with the atmosphere, reflects in its behavior the varying atmospheric conditions as regards humidity. The sample would deteriorate more rapidly than the original, when the latter is exposed to a dry atmosphere, from which the sample would be protected from loss of moisture by virtue of the tightly fitting top to its container.

A significant question arises in connection with the consideration of the deterioration of sugar samples in storage. It is quite natural to assume that there must be a limit at which deterioration automatically ceases. But what is this limit, and what conditions regulate it? Thus Browne[2] finds the limit at which raw cane sugars cease deteriorating to be somewhat lower than indicated by the factor .333, and the ratio was in all cases approximately .250. Thus, to cite an example given by Browne of the limiting factor in sugar deterioration, we have the following:

TABLE IV.

	Muscovado No. 1.	Muscovado No. 2.
Polarization—August, 1910	92.70	92.65
" October, 1914	87.30	87.70
" January, 1915	87.25	87.60
Analysis—January, 1915, Water	3.04	2.88
" Sucrose Clerget	88.30	88.57
" Invert Sugar	6.25	6.31
" Ash	0.73	0.77
" Undetermined	1.68	1.47
" Ratio Water / 100-Sucrose	0.260	0.252

[1]Loc. cit
[2]Loc. cit.

The explanation of the automatic cessation of deterioration in sugar samples, which are prevented from absorbing moisture from the atmosphere, when a certain solid non sucrose ratio is reached, is in the writer's opinion to be sought for in the absence of sucrose in the molasses film, rather than in the influence of autointoxication on the part of the micro-organisms involved. In this view the writer concurs fully with Browne,[1] who, however, uses as a partial explanation of the phenomenon, the results of the experiments of Brain and Deerr[2] upon the action of deteriorative species of bacteria upon 10% sucrose solution. In the experiment referred to the authors noted that while all of the cane sugar in this inoculated solution was transformed in a very few days, the destruction of the reducing sugar does not progress beyond a certain stage, and remained constant after the tenth day. In the writer's opinion, it was not the toxic substances that inhibited the further destruction of reducing sugars by the micro-organisms, but the exhaustion of the nutrient element essential to the further development. The formula for the 10% sucrose solution employed in the experiments under consideration contains very little nitrogen, in fact, carrying only 0.1 peptone. Furthermore, for these micro-organisms sucrose is a much more readily utilizable source of energy than is either dextrose or levulose. This fact is shown in experiments of the writer[3] where in various mixtures of sucrose and dextrose and levulose singly and combined, the destruction of sucrose was always more rapid than that of the other sugars. It is to be expected, therefore, that the destruction of reducing sugar would be extremely slow in view of the exhausted state of the nutrients in the solution, after the fermentation of the sucrose had been completed.

We come next to consider the interesting observation previously cited by Browne[4] and confirmed by the writer's experiments, that, although the average 96 test sugar will not deteriorate in storage so long as its moisture non sucrose solids ratio is .333, yet once a sugar begins to deteriorate it will continue to decrease in polarization until the factor falls to approxi-

[1]Loc. cit.
[2]Brain & Deerr, Bact. Flora of Hawaiian Sugar Bul. 9, Hawaiian Experiment Station.
[3]La. Bul. No. 125.
[4]Loc. cit.

mately 0.250. To explain this fact it is necessary to regard this solid non sucrose ratio in the light of the varying density of the molasses to which it applies. The validity of this factor would seem to depend in most cases upon the fact that it connotes a certain density of the molasses film surrounding the sugar crystals. Generally speaking, the molasses films of unwashed sugar are of a density corresponding to the limit of saturation for a product of its purity. Hence a high ratio of solids non sucrose to moisture indicates a low purity molasses, and a corresponding tendency, according to the melassegenic theory of Geerligs[1] to resist crystallization at high densities. The validity of the factor of safety depends upon the fact that it connotes a density of film at which the sucrose destroying power of microorganisms is no longer possible. This applies only, of course, to natural molasses films. Now in most of the sucrose destroying types of fermentation which take place in the film, the sucrose is directly inverted, and only a small portion of the reducing sugar formed is utilized, as a result of which no material alteration of the density of the films of molasses takes place. So while the validity of the factor of safety for an undeteriorated sugar depends upon the fact that it connotes a film of such a density that inversion of sucrose cannot take place, the meaning is quite different with a deteriorated sample. In the latter case we may have a sugar in which the moisture content remains constant, but in which the ratio of non sucrose solids to moisture gradually increases by virtue of the inversion of sucrose. Obviously this gradual change from an unsafe to a safe ratio of solids non sucrose to moisture need not indicate any decreased tendency to deteriorate except that it suggests a decrease in the amount of sucrose in the film. Hence in this case the deterioration, which begins in a film of a density under the maximum for the inversion of sucrose, may result in the complete destruction of all the sucrose in the film. And this takes place in spite of the fact that the course of the fermentation proceeds through stages of solid non sucrose-moisture ratios, which would have been prohibitive of fermentation changes had they applied to the original sugar. However, it must not be inferred from this that the cessation of

[1]Prinsen Geerligs Cane Sugar and its manufacture, p. 305.

deterioration is always coincident with the exhaustion of the sucrose in the molasses film. On the contrary, Browne[1] has shown that the complete destruction of all of the sucrose in the molasses film of a deteriorating sugar, in a sealed sample, rarely ever occurs. The fact that deterioration ceases when sucrose still remains in the film finds a possible explanation in the fact that the invert sugar formed from the sucrose gives the solution considerably higher osmotic pressure, and hence has the same effect as an increase in the density of the film.

[1]Chemical Factor in the Deterioration of Raw Cane Sugar.

TABLE VI.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
1	P. G.	3/20/16	Cont.	99.50	99.84	99.51	99.85	0.02	—	.040	420
	"	7/26/16	"	98.80	98.98	98.88	99.06	0.20	—	.075	420
3	P. G.	3/20/16	Inoc. Bact.	99.20	99.23	99.24	99.27	0.05	—	.0625	1185
	"	7/26/16	"	98.90	99.30	99.07	99.49	0.18	0.23	.163	1185
5	P. G.	3/20/16	Inoc. Yeast	99.65	99.85	99.70	99.90	0.06	—	.171	160
	"	7/26/16	"	98.80	99.48	99.01	99.59	0.12	—	.100	160
15	Y. C.	3/20/16	Cont.	97.80	97.84	98.05	98.09	0.26	1.32	.118	8250
	"	7/26/16	"	96.80	97.57	97.18	97.96	0.40	1.80	.124	8250
16	Y. C.	3/20/16	Inoc. Bact.	97.60	98.33	98.04	98.77	0.45	1.18	.187	2225
	"	7/26/16	"	96.20	96.87	96.78	97.45	0.60	2.15	.157	2225
17	Y. C.	3/20/16	Inoc. Yeast	97.80	98.48	98.06	98.74	0.27	1.53	.122	8050
	"	7/26/16	"	96.20	97.03	96.78	97.61	0.60	2.22	.157	8050
18	La. 2nds	3/20/16	Cont.	84.00	84.57	86.33	—	2.70	5.26	.168	7650
	"	7/26/16	"	82.40	83.63	85.26	86.64	3.36	4.76	.190	7650
20	La. 2nds	3/20/16	Inoc. Bact.	84.00	85.38	86.41	87.83	2.80	5.13	.112	1467
	"	7/26/16	"	82.40	83.94	85.45	87.05	3.58	4.88	.203	1467

TABLE VII.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
24	La. 2nds	3/20/16	Inoc. Yeast	84.20	85.64	86.42	87.92	2.60	5.13	.164	700
	"	7/26/16	"	82.80	84.25	85.36	86.80	3.00	4.76	.174	700
2	La. 96	3/20/16	Cont.	96.00	96.41	97.11	97.53	1.15	1.25	.287	1340
	"	7/26/16	"	95.50	95.73	96.77	97.01	1.32	1.06	.293	1340
4	La. 96	3/20/16	Inoc. Bact.	95.90	96.17	96.85	97.13	0.99	1.25	.241	495
	"	7/26/16	"	95.90	96.50	97.39	97.99	1.53	0.89	.373	495
6	La. 96	3/20/16	Inoc. Yeast	96.20	96.72	97.31	97.84	1.14	1.13	.300	545
	"	7/26/16	"	95.80	96.59	97.15	97.94	1.39	1.06	.330	545
7	La. 96° Washed	3/20/16	None	95.50	95.85	96.72	97.06	1.25	1.66	.277	525
	"	7/26/16	"	94.85	95.92	95.94	97.02	1.14	1.51	.221	525
19	La. 96°	3/20/16	Cont.	96.60	97.29	97.39	98.09	0.82	1.45	.241	1560
	"	7/26/16	"	95.35	96.09	96.24	96.99	0.93	1.41	.200	1560
21	La. 96°	3/20/16	Inoc. Bact.	96.20	96.45	97.11	97.36	0.94	1.48	.247	1635
	"	7/26/16	"	95.10	95.83	96.20	96.94	1.05	1.39	.214	1635
26	La. 96°	3/20/16	Inoc. Yeast	96.00	96.18	96.77	.....	0.80	1.64	.200	1243
	"	7/26/16	"	94.50	95.17	95.38	96.06	0.93	1.41	.169	1243

TABLE VIII.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
11	La. 2nds	3/20/16	None	82.20	83.36	87.91	89.15	6.50	3.17	.365	810
	"	7/26/16	"	80.00	82.37	85.45	87.98	6.38	4.35	.319	810
36	La. 2nds	3/20/16	Cont.	90.20	90.26	93.08	93.14	3.10	2.15	.316	855
	"	7/26/16	"	87.70	88.40	90.22	90.94	2.80	2.44	.232	855
37	La. 2nds	3/20/16	Inoc. Bact.	90.40	90.74	93.31	93.66	3.12	1.62	.325	18,100
	"	7/26/16	"	89.10	89.69	91.54	92.08	2.60	2.32	.238	18,100
42	La. 2nds	3/20/16	Inoc. Yeast	90.20	90.76	93.44	94.02	3.47	1.62	.354	2,157
	"	7/26/16	"	89.70	90.31	92.09	92.70	2.68	2.27	.252	2,157
29	P. G.	3/20/16	Cont.	99.30	99.91	99.37	99.89	0.08	—	.114	632
	"	7/26/16	"	98.80	99.32	98.91	99.43	0.12	—	.100	632
35	P. G.	3/20/16	Inoc. Bact.	99.40	99.65	99.44	99.69	0.05	—	.0833	670
	"	7/26/16	"	98.30	98.86	98.56	99.12	0.27	0.34	.158	670
41	P. G.	3/20/16	Inoc. Yeast	99.40	99.52	99.62	99.74	0.23	—	.383	195
	"	7/26/16	"	98.50	98.86	98.69	99.05	0.20	0.27	.133	195

TABLE IX.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
9	96° T. Washed	1/8/16	Cont.	97.70	97.78	98.33	98.42	0.65	0.95	.239	1,135
	"	7/26/16	"	95.70	96.15	96.39	96.84	0.72	2.22	.167	1,135
10	96° T. Washed	3/20/16	Washed with 2 1.1.% Na2 HPO4	96.30	96.88	97.42	98.00	1.15	1.41	.310	1,230
	"	7/26/16	"	93.45	94.15	94.72	95.43	1.35	3.03	.206	1,230
8	96 T. Washed	3/20/16	None	96.50	97.13	97.57	98.21	1.10	1.66	.314	610
	"	7/26/16	"	93.50	94.31	94.62	95.44	1.19	3.22	.183	610
27	96 T. Washed	3/20/16	Cont.	96.80	96.81	97.69	97.70	0.92	1.04	.287	2,507
	"	7/26/16	"	94.30	94.77	95.46	95.94	1.22	1.41	.214	2,507
31	96 T. Washed	3/20/16	Inoc. Bact.	97.00	97.58	98.07	98.56	1.10	0.85	.366	16,880
	"	7/26/16	"	95.20	95.56	96.52	96.88	1.37	1.72	.275	16,880
34	96 T. Washed	3/20/16	Inoc. Yeast	96.90	97.05	98.07	98.22	1.20	0.82	.387	920
	"	7/26/16	"	94.75	95.31	96.10	96.67	1.41	2.00	.268	920
23	La. 96 T.	3/20/16	Cont.	94.00	94.27	96.06	96.34	2.15	1.71	.358	1,650
	"	7/26/16	"	91.40	92.30	93.40	94.32	2.15	2.32	.250	1,650
30	La. 96 T.	3/20/16	Inoc. Bact.	93.40	93.83	95.47	95.91	2.17	1.61	.328	709
	"	7/26/16	"	92.20	93.05	94.56	95.43	2.50	2.32	.320	709
33	La. 96 T.	3/20/16	Inoc. Yeast	93.40	93.41	95.71	95.75	2.42	1.61	.366	712
	"	7/26/16	"	91.85	92.45	93.15	93.76	2.47	2.50	.303	712

TABLE X.

Table Showing Changes in Composition of Inoculated Sugar in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
12	La. 96 T. Washed	3/20/16	Cont.	96.90	97.69	98.42	99.22	1.55	0.92	.500	2,200
	"	7/26/16	"	94.35	95.03	95.72	96.41	1.44	2.44	.254	2,200
13	La. 96 T. Washed	3/20/16	Washed 1 L. 5% Na_2HPO_4	96.80	97.53	97.77	98.51	1.00	0.80	.312	3,630
	"	7/26/16	"	94.70	95.52	95.85	96.68	1.21	1.82	.228	3,630
14	La. 96 T. Washed	3/20/16	Washed 1 L of 1% Na_2HPO_4	96.20	96.47	97.56	97.83	1.40	1.11	.368	5,407
	"	7/26/16	"	93.50	94.27	94.84	95.62	1.42	2.50	.218	5,407
22	P. G. undried	3/20/16	Cont.	97.10	97.35	98.52	98.77	1.45	0.32	.486	987
	"	7/26/16	"	96.10	96.61	97.27	97.79	1.21	1.48	3.10	987
25	P. G. undried	3/20/16	Inoc. Bact.	97.00	97.32	98.67	99.00	1.70	0.36	.566	31,675
	"	7/26/16	"	95.80	96.45	97.93	98.59	2.18	1.21	.517	31,675
28	P. G. undried	3/20/16	Inoc. Yeast	97.00	97.32	98.87	99.20	1.90	0.33	.633	81,490
	"	7/26/16	"	95.30	95.80	97.34	97.85	2.10	1.33	.447	81,490
32	P. G.	3/20/16	Cont.	99.80	99.95	99.81	99.96	0.02	—	.100	172
	"	7/26/16	"	99.05	99.60	99.09	99.64	0.05	—	.052	172
	P. G.	3/20/16	Inoc. Bact.	99.80	99.85	99.96	100.00	0.16	.....	.800	1,360
	"	7/26/16	"	98.85	99.29	99.06	99.50	0.22	0.32	.103	1,360
44	P. G.	3/20/16	Inoc. Yeast	99.70	99.81	99.87	99.99	0.18	—	.600	202
	"	7/26/16	"	99.10	99.24	99.18	99.32	0.09	—	.100	202
45	Y. C. undried	3/20/16	None	95.80	96.42	99.44	100.00	3.67	0.88	.873	4,000
	"	7/26/16	"	94.10	94.39	96.30	96.60	2.29	2.12	.388	4,000
46	Y. C. undried	3/20/16	None	95.80	95.91	97.91	98.02	2.16	1.31	.514	2,007
	"	7/26/16	"	92.80	93.37	94.59	95.17	1.90	3.12	.263	2,007

SECOND SERIES OF SAMPLES.

In the experiments with the first series of sugar samples under observation in storage, the results of which have been discussed, the plan was mainly to observe the relative susceptibilities of various types of sugar to deterioration, and to note the effects of high temperatures and relatively high humidities upon those changes.

In the experiments carried on with sugar samples the following year, an effort was made to determine, as far as possible, the influence of the degree of infection of sugar upon their deterioration. The samples were procured in much larger quantities than in the previous year, and they were thoroughly mixed upon their arrival at the laboratory, and divided into three equal portions. These triplicate samples were then treated as follows: One was inoculated with a one cc portion of a 24-hour growth of a culture of bacteria obtained from sugar. Another was inoculated from a molasses agar streak of a torula culture, and to the third was added one cc of sterile distilled water. The addition of the liquid was made in both cases with an atomizer inoculator as described by the writer in a former publication.[1] After treatment the samples were again thoroughly mixed and placed in fruit jars and kept for further observation. The first analysis of the samples was not made until a month after the inoculation, in order that an opportunity might be allowed for the effect of the treatment to be reflected in the initial analysis.

A DISCUSSION OF RESULTS.

An examination of the analytical data upon the second series of sugar samples during storage shows few cases where the inoculation may be credited with any effect upon the changes in the composition of the samples. Even in those rare cases, where the inoculation seemed to bear results in an accelerated deterioration, the moisture content of the inoculated samples was more favorable for deterioration than was that of the controls. Moreover, the quantitative bacteriological analysis of the samples, which was made a month after the inoculation, did not frequently show an increase in the degree of infection of those samples over

[1]Bulletin 125, La. Experiment Station

the controls. The negative nature of the results of inoculation, as shown in these experiments, indicated that in most cases sugars contain the necessary deteriorative potential of micro-organisms, and when no deterioration takes place it is more often than otherwise due to the lack of suitable conditions for their development.

The results of these experiments show a much larger percentage of deterioration in the samples than in the previous series. This is no doubt due to the fact that in the present series there is a much larger percentage of washed 96 test and plantation white sugar. These types of sugar are, as we have already observed, more susceptible to deterioration than their ratio of solids non sucrose to moisture would indicate. The added moisture incident to the inoculation no doubt had an accelerative action upon the deterioration of the samples, even if it did not appear to endanger their keeping qualities as judged from the factor. In the case of the second sugars with a high factor, there does not seem to be the same tendency for them in this series to lose only in reducing sugars, as was observed in the previous series of samples. We find an indication of this type of change in composition during storage only in the case of Sample No. 24. Sample No. 36, for example, deteriorated with a factor of .316, and Sample No. 11 with a factor of .365.

REGARDING THE NUMBER OF MICRO-ORGANISMS IN SUGAR, AND QUANTITATIVE METHODS FOR THEIR DETERMINATION.

We come next to a consideration of the number of micro-organisms in sugars with special reference to the adequacy of these numbers to account for the changes which sugars undergo in storage. Experimental data upon this subject indicates that the number of micro-organisms in sugars is not a reliable criterion of the tendency of sugars to deteriorate. As will be noted from the preceding tables, the bacteriological counts of sugars often show the highest degree of infection in those samples which did not deteriorate. Furthermore, these counts are below what would be expected from any ordinary substance undergoing

chemical changes as the result of biological activities. It has been objected to the theory of the biological cause of sugar deterioration, that the number of micro-organisms shown in bacteriological analyses of sugars are too small to account for any detectable changes in their chemical composition. According to the work of Rahn[1] at least one million living cells of bacteria per cc. are necessary to produce any measurable changes in the composition of any substance. In sugars this number of micro-organisms per gram is rarely ever found. The writer has very rarely found sugar containing over 500,000 micro-organisms per gram. Deerr and Norris[2] report a variation in the degree of infection of sugar samples ranging from less than 100 cells per gram to 10,000. In many cases they report the number occurring in sugars with the sign of infinity, but in these cases it is likely that the excessive number refers to an overcrowding on the agar plates due to the use of too low a dilution of the sugar examined. Greig Smith[3] also reports a very small number of micro-organisms per gram in sugars that he examined, although he appears not to have made very extensive quantitative determinations.

There is one factor which has to be taken into account when interpreting the meaning of a quantitative bacteriological analysis of sugars. In dealing with sugars we are dealing with a substance which, from a bacteriological standpoint, consists of a large amount of inert and a small amount of infected material. The sugar crystal is, in all probability, a sterile substance within its interior, and the infection of its exterior is limited to the amount of molasses it carries as its film. This being the case, a quantitative bacteriological estimation of the number of micro-organisms in sugar really gives the number contained in the film of molasses surrounding the crystals. And since the ratio of molasses to sugar crystal is not always known, the number of micro-organisms as ascertained by bacteriological analysis refers to a dilution of unknown magnitude. The ratio of molasses to crystal may vary possibly from 0.1 to 10 per cent. Therefore the high counts obtained from low grade sugars may not be rela-

[1]The fermenting capacity of the average single cell of Bacterium lactis acidi, Otto Rahn, Michigan Technical Bulletin, No. 10.

[2]Loc. cit.

[3]The Deterioration of Raw and Refined Crystals in Bulk, I. S. J., Vol. IV, 1902, pp. 430, 433, 481, 483.

tively higher for the film than the low counts obtained from high grade sugars. There is no doubt, also, that the difficulty of elaborating adequate quantitative methods has resulted in obtaining erroneously low counts in the bacteriological analyses of sugars. Owen[1] showed that the 10% sucrose agar so generally used for the quantitative bacteriological analysis of sugar gave much lower results than media of higher densities. The use of higher density media resulted in fact in showing the presence in large number of torula in sugars. The higher counts obtained from these media were almost entirely due to the fact that they admitted of the development of this group of micro-organisms, which had been almost entirely suppressed by the prejudicial osmotic effects of the low density media. The writer, however, has recently had occasion to make further observation on the subject, which shows that the principle of high density media must be carried still further to become most effective. The occasion referred to was the desire to ascertain the number of micro-organisms in fermenting cane syrup. In those experiments the counts from syrups which were undergoing vigorous fermentation appeared entirely too low, although the medium employed in the determinations was a 30° Brix molasses agar. In the quantitative bacteriological analysis of sugars the number of micro-organisms that would be expected to be present even in rapidly deteriorating samples is very indefinite, as we have noted, owing to the unknown ratio between film and crystal. But in dealing with syrups or molasses we would expect to have a development on the plates of a number of micro-organisms commensurate with the changes in chemical composition which the material is undergoing. In the case of vigorously fermenting syrups or molasses we could not feel satisfied with a count of a few thousands, or even a few hundred thousands per gram. But at the beginning of our experiments, with the use of a high density medium and dilution water, we were obtaining just such inadequate numbers in the analysis of fermenting syrup.

After various experiments with modification of the culture medium, which consisted chiefly of varying its titre and density, it was thought advisable to substitute a syrup solution for the

[1]Bacteriological Investigations of Cane Sugar Products, La. Bulletin 146.

water in making the dilution for the plates. The results of the experiment, in which a 30° Brix syrup solution was used in comparison with water, in making the dilution for the plates, are given in the following table:

Table Showing a Comparison between the Estimation of the Number of Micro-organisms in Syrups and Sugars by the use of Water and Syrup Solution Methods of Dilution:

SAMPLE	NUMBER PER GRAM.	
	Water Dilution	Syrup Dilution
Syrup No. I	83,803	1,838,250
" No. II	106,500	1,465,000
" No. III	467,500	1,828,750
" No. IV	450,000	1,053,500
" No. V	5,100	373,350
" No. VI	15,250	2,218,500
" No. VII	297,000	1,520,000
" No. VIII	5,928	160,000
" No. IX	69,160	765,000
" No. X	.....	1,187,500
" No. XI	732,500	1,184,300
" No. XII	9,550	785,230

Medium—30 Brix Syrup Agar.

The comparison between the counts obtained with the use of water and with those obtained with the syrup solution is very striking. In these experiments a careful check was made of the sterility of the syrup solution. The fact that no growth was ever obtained in plates to which the uninoculated dilution syrup was added showed that none of the higher counts obtained by this method could be accredited to the contamination of the dilution syrup. The explanation of the higher counts obtained by this method is no doubt due to the same causes which were responsible for the higher counts that were obtained with the use of high density culture media compared with media of low density.[1]

We have already offered as an explanation of the higher counts obtained in the quantitative bacteriological analysis of syrups with the substitution of syrup solution for the dilution water ordinarily used, that the former method eliminated the prejudicial effect upon the torula of the sudden change of density. If this explanation is correct, then the advantages in favor of the syrup dilution method over the water dilution method should increase in proportion to the length of the exposure of the micro-organisms to these different diluent fluids.

[1]Loc. cit.

A test of this hypothesis was made in the following experiment. In this experiment the syrup to be analyzed was weighed into sterile measured portions of water and a 30° Brix syrup solution. Transfers from the flasks thus inoculated were made to plates immediately and after a period of two hours:

Table showing the comparative counts on syrups and sugars with the use of water and syrup solution method.

SAMPLE	WATER SOLUTION		30 BRIX SYRUP SOLUTION	
	Immediate Transfer	Transferred After 2 Hours	Immediate Transfer	Transferred After 2 Hours
Fermented Syrup	17,444	7,314	1,300,000	1,050,000
Sugar (a) Inoculated with torula	712,500	390,000	4,120,000	4,325,000
Sugar (b) inoculated with torula	737,500	63,250	2,967,0 0	1,980,000

Medium—30 Brix Syrup Agar.

The results of this experiment show that there is a great decrease in the number of micro-organisms developing on the plates inoculated from the water dilution, following an exposure of two hours. On the other hand, the result of a similar exposure to the syrup solution did not show any constant tendency either to increase or decrease. These results disprove as a possible explanation of the high counts on the syrup dilution plates, the suggestion that an increase in the number of micro-organisms takes place in the diluent solution. The use of water as a diluent fluid for the quantitative estimation of the micro-organisms in syrups or sugars obviously yields results that are misleading, since it depresses in a large measure the development of torula upon the plates. In this case we have an instance where the method of obviating the plasmolyzing effects of water as a diluent for quantitative bacteriological determinations, by the use of solutions containing a relatively large amount of solids, finds its logical application. It is interesting to note in this connection that this principle was once suggested as a modification of the water diluent method in the quantitative bacteriological determination of soils. The further investigation of this interesting observation might prove profitable from the standpoint of scientific interest. It is regretted by the writer that the lack of time prevented a more complete investigation of it. It would be particularly interesting, for example, to determine the rate of decrease in counts during certain intervals of time of exposure. Whether or not the rate of decrease would give a curve of regular or irregular slope is a question whose answer must await further investigation.

PART II.

THE MICRO-ORGANISMS CONSTITUTING THE CAUSATIVE AGENCIES IN THE CHANGES IN THE COMPOSITION OF SUGARS IN STORAGE.

A.

Bacteria.

The characteristics of the species of bacteria occurring in sugars were rather closely studied in a previous investigation of sugar deterioration, the results of which appeared in Bulletin 125. In the present investigation an effort was made to subject the various bacterial cultures isolated from sugars to a more comprehensive study, the purpose of which was to determine whether the different characteristics of the cultures were fixed, or whether they represented temporary adaptations to specific environments. The work of isolating the cultures was also conducted on a much broader basis than in the previous investigation. In the present case due significance was accorded to the idea of the possibility of the occurrence in sugars of certain species of bacteria which might not develop under the ordinary conditions of cultivation. It was therefore decided to vary the cultural conditions as broadly as possible in the attempt to isolate all species of any possible economic importance. With this in view, a series of culture media was substituted for the one medium previously used. In addition, moreover, to varying the media, the inoculated plates were exposed to both aerobic and anaerobic conditions, and to different degrees of temperature in incubation. It was thought that a combination of these various cultural conditions would result in the isolation of all of the species occurring in sugars. This work was carried on for several months, and although it resulted in a large number of cultures being obtained, yet these were reduced by successive cultivations under identical conditions to eighteen. Anaerobic and aerobic conditions, as well as low and high incubation temperatures, tend to yield quantitative rather than qualitative differences.

Having once reduced the number of cultures to what appeared to be an irreducible minimum, the work was continued further and the cultures used were grown for a number of generations upon the same medium to see if the differentiating cultural characteristics of the various cultures could be still further merged. It was found that the characteristic colony formation of the various cultures, after having been grown for a number of successive generations, under identical cultural conditions, did vary considerably from the original. However, in spite of the fact that all of the cultures did vary, yet the variation for each culture seemed to be distinctive. Hence the distinctiveness of the various cultures was as pronounced under the conditions of successive cultivations under identical conditions, as when first isolated from sugars.

Relationship to the Potato Group of Bacteria.

It was Greig Smith[1] who first pointed out the similarities of the species of bacteria occurring in sugars to the potato group of organisms. The most striking points of resemblance between the two groups are the very high resistance of their spores to heat, the ability to form gum in the fermentation of certain sugars, as well as in their general morphological and physiological characteristics. For the purpose of convenience in comparing the characteristics of the two groups, the data is condensed in the following table:

[1]Vol. XXVI Linnear Society of New South Wales.

TABLE I.

Table Showing Characteristics of Species of Bacteria Isolated from Sugars in Comparison with Potato Species.

Culture	Size in	Flagella	Grams' Stain	Reaction to Oxygen		Liq. of Gel.	Bouillon Cult.		Spore Formation	Milk Cult.		Indol. Reaction	H_2S Product 10d	Acid from 2% Dextrose	Gas from any Sugar	Characteristics Exp. Numerically
				Aerobic	Anaerobic		Film	Cloudy		Coag.	Reaction					
Bac. vulgatus.....	L1-M6-5.0 B-0.8.....	+	+	+	+	+	+	+	+	+	Alk.	–	+	2.3	–	121.2025
Bac. mesentericus.	L-0.8-2.4 B-0.7-2.9	+	+	+	+	–	–	+	+	+	Alk.	T	+ +	3.00	–	122.2020
B. lioder mos.....	L3.5 B1.2	+	+	+	–	+			+	+					–	121.2020
B. mes. ruber.....	L-1.4 B-0.4.....	+	+	+	+	+			+	–					–	121.2020
Cult. I.....	L-4.7 B-1.0.....	+	+	+	+	+	+	+	+	+	Acid	T	+ +	2.4	–	121.2025
Cult. II.....	L-3.0 B-1.4.....	+	+	+	+F	+	+	+	+	+	Acid	T	–	2 4	–	121.2025
Cult. III....	L-2.8 B-2.0.....	+	+	+	–	+S	+	+	+	+S	Acid	T	–	2.4	–	111.2020
Cult. IV.....	L-3.4 B-1.7.....	+	+	+	+	+S	+	+	+	+S	Acid	T	+ +	1.7	–	121.2025
Cult. V.....	L-3.3 B-1.4.....	+	+	+	+	+S	+	–	+	+S	Acid	T	+ +	2.3	–	121.2025
Cult. VI.....	L-4.9 B-1.4.....	+	+	+	+F	–	+	+	+	+S	Acid	T	+	1.88	–	122.2025
Cult. VII....	L-3.9 B-1.1.....	+	+	+	+F	+S	+	+S	+	+S	Acid	T	+ +	2.00	–	121.2025

TABLE I---Cohtinued.

Culture	Size in M.	Flagella	Grams' Stain	Reaction of Oxygen		Liq. of Gel.	Bouillon Cult.		Spore Formation	Milk Cult.		Indol. Reaction	H_2S Product 10d	Acid from 2% Dextrose	Gas from any Sugar	Characteristics Exp. Numerically
				Aerobic	Anaerobic		Film	Cloudy		Coag.	Reaction					
Cult. VIII...	L-3.4 B-1.2.....	+	+	+	+	+S	–	+S	+	+S	Acid	T	+ +	2.2	–	121.2025
Cult. IX....	L-4.0 B-1.5.....	+	+	+	+	+S	+S	+	+	+S	Acid	T	+ +	2.3	–	121.2025
Cult. X.....	.....	+	+	+	+	+VS			+	+S	Acid		+	2.1	–	121.2025
Cult. XI....	L-3.7 B-1.2.....	+	+	+	+	+S	–	+	+	+S	Acid	T	+ +	2.1	–	121.2025
Cult. XII....	L-4.0 B-1.8.....	+	+	+	+		–	+	+	+S	Acid	T	+ +	2.3	–	121.2025
Cult. XIII...	L-3.2 B-1.4.....	+	+	+	+	+S	–	+	+	+S	Acid	T	+	1.85	–	121.2025
Cult. XIV...	L-3.4 B-1.3.....	+	+	+	+	+S	+	+	+	+S	Acid	T	+ +	1.7	–	121.2025
Cult. XV....	L-3.8 B-1.2.....	+	+	+	+	+S	+	+	+	+S	Acid	T	+	2.7	–	121.2020
Cult. XVI...	L-3.5 B-1.9.....	+	+	+	+	+S	+	+	+	+S	Acid	T	+	1.88	–	121.2025
Cult. XVII..	L-2.2 B-1.0.....	+	+	+	+	+VS	+	+	+	+S	Acid	T	+	1.88	–	121.2025
Cult. XVIII.	diam. 1.0	–	+	+	+F	+VS	–	+	–	+S	Acid	T	+ +	3.6	–	221.2020

T Trace
\+ Moderate.
\+ } Abundant.
\+ }
All acidities in milk very slight.

S—Slow.
V. S.—Very Slow.
F.—Feeble.

A critical examination of the above table shows that each of the various cultures obtained from sugars tends to conform to one or the other of the principal species of the potato group of bacteria. Just as we find liquefaction and non-liquefaction of gelatin as the respective characteristics of two of the species of the potato group, so we find the same variation in this respect in the culture obtained from sugars. In size as well as in the ability to liquefy gelatin, the majority of cultures from sugars show a closer relationship to *Bac. vulgatus* than to *Bac. mesentericus*. And this similarity also agrees to a certain extent with the relative sucrose destroying power of the different cultures. Rapid gelatin liquefaction seems usually to be associated with a strong sucrose destroying ability on the part of the cultures.

In the numerical system[1] of recording the characteristics of bacterial species, which we have used in the last column of our table, we have applied the decimal ending .0005 to gum fermentation of sucrose, and the ending .0000 to signify the lack of this ability on the part of the cultures. It will be noted that culture III, culture XV and culture XVIII lack the ability to induce the gum fermentation in sucrose solutions. These three cultures appear to take no part in the deterioration of sugars, nor do they seem to have any appreciable influence upon sucrose solutions. Their frequent occurrence in raw sugars, however, made a study of their cultural and physiological characteristics of some importance. These three species constitute the principal inert species of bacteria in sugar. Greig Smith[2] describes the inert bacteria in the Australian sugars as belonging to the streptothrix group. The term inert is used in this connection to indicate a normal rather than an abnormal state of these species. It refers to the inability of the species to cause the deterioration of sugars, even under the most favorable conditions.

Culture III and culture XV exhibit most of the characteristics of the potato group of bacteria. The former is a short, thick bacillus, the latter conforms in size to the average cells of *Bac. vulgatus*. Neither III nor XV developed any gum-forming power in the course of successive development in sucrose solu-

[1]Chart of the American Association of Bacteriologists.
[2]Loc. cit.

tions. Culture III appears to be a strict aerobe, as no growth from it has been obtained under anaerobic conditions. Culture XVIII is a small micrococcus of 1 c.c. diameter. The frequency with which these species occur in sugars suggests a resistance on their part to plasmolyzing influence of high density solutions. The presence of a micrococcus in beet sugars was noted by A. Schone,[1] who claimed that this group constituted the largest part of the microflora of these sugars.

The identity of the species of bacteria in sugars with the potato group of bacteria is clearly indicated in the morphological and physiological characteristics of the individual species in the two groups. The supposition seems also to admit of easy confirmation, since we have had no trouble in isolating from the soil, in the field at a distance from the sugar factory, cultures of bacteria which would conform in characteristics to the potato group, and would at the same time induce the characteristic gum fermentation of sucrose. But the conditions of those experiments did not preclude the possibility of the species thus isolated from the soil having been previously acclimatized to sugar solutions at the sugar factory and subsequently reintroduced into the soil. To obviate this possibility, pure cultures of the potato species of bacteria were obtained from the Museum of Natural History of New York City, through the courtesy of the curator, Dr. C. E. A. Winslow. These cultures were transferred to flasks of sterile 10 per cent sucrose solution to compare their sucrose destroying ability with that of a culture isolated from sugars. At the same time transfers were made from the museum cultures to tubes of 10% sucrose solution for the purpose of acclimatizing these cultures to sugar solutions. Transfers from the inoculated tubes of sucrose solutions to test tubes were made at the end of every 48 hours. After one week of this acclimatization to 10% sucrose solution these cultures were used to inoculate a second set of flasks. A second acclimatization period preceded the third experiment, so that the cultures used in this experiment had been grown for two weeks in a ten per cent sucrose solution and had been brought into contact with fresh medium every 48 hours. Coincident with this acclimatization of the cultures of the potato species to sugar solutions, a reverse process was conducted

[1]Deutsche Zuckerindustrie, 1906 Bd. 31, S. 1837.

whereby an attempt was made to lower the sucrose destroying power of a species isolated from sugar, by growing it for successive generations in bouillon.

The following table shows a comparison of the sucrose destroying power of unacclimatized cultures of the potato group of bacteria with that of a culture isolated from sugars.

TABLE II.

PART II---BACTERIA.

Table Showing Ability of Potato Species Isolated from Sources other than Sugar Products, to acquire the power of destroying Sucrose.

Smith's 10% Sucrose Solution.

Cult.	Brix	Sucrose		1st Inoculation		Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase in R. S.
		S. P.	S. C.	Reducing Sugar	Viscosity			
Control.....	11.22	9.80	9.72	0.09	1.34	0.40	—	—
B. mesentericus...	11.52	9.50	9.49	0.10	1.33	0.84	0.30	0.01
B. liodermus.....	10.98	9.50	9.40	0.15	1.33	0.80	0.30	0.06
B. mes. vulgatus...	9.98	5.40	Gum	4.17	1.77	0.60	4.40	4.08
B. mes. ruber.....	10.98	8.60	Gum	1.02	1.60	1.00	1.20	0.93
Cult X. Bouillon...	9.98	1.00	Gum	5.70	3.30	0.80	8.80	4.61
Cult X.10% SS sol.	10.36	1.30	Gum	5.70	3.27	0.80	8.50	3.18

2 Weeks Inoculation.

Second Inoculation with Cultures Grown for one Week in S. S.

Transferred Every 48 Hours.

Cult.	Brix	Sucrose		Reducing Sugar	Viscosity	Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase in R. S.
		S. P.	S. C.					
Control.....	10.43	9.50	9.64	—	1.26	0.30	—	—
B. liodermus.....	10.49	9.50	9.64	—	1.25	0.50	—	—
B. mesentericus...	10.57	9.30	9.44	—	1.26	0.90	0.20	—
B. mes. ruber.....	10.57	3.00	Gum	6.89	2.23	0.90	6.50	6.89
B. vulgatus.....	10.33	0.40	Gum	7.54	2.77	0.60	9.10	7.54
Cult. X 10% S. S..	10.33	0.95	Gum	10.80	3.53	0.60	9.05	10.80
Cult. X. Bouillon..	10.63	1.10	Gum	11.00	3.21	0.80	8.40	11.00

TABLE III.

PART II A.---BACTERIA.

Table showing ability of potato species of bacteria isolated from sources other than sugar products to acquire the power of destroying sucrose.

(3rd inoculation after two weeks transferring.)

Cult.	Brix	Sucrose		Reducing Sugar	Viscosity	Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase R. S.
		S. P.	S. C.					
Control.....	10.43	9.40	9.56	—	1.25	0.1	—	—
B. mes.....	10.61	9.35	9.21	—	1.25	0.95	0.05	—
B. liodermos.....	10.16	9.30	9.56	—	1.25	0.60	0.10	—
B. mes. ruber.....	10.19	2.90	Gum	6.55	3.70	0.70	6.50	6.55
B. vulgatus.....	10.19	0.80	Gum	7.54	3.02	0.80	8.60	7.54
Cult. X. S. S.....	9.49	1.00	Gum	7.40	3.08	0.80	8.40	7.40
Cult X. Bouillon...	10.23	0.60	Gum	7.69	3.08	0.60	8.80	7.69

Summary of data on the previous Experiments upon the acquired Sucrose Destroying Powers of the Potato Species.

Cult.	1st Experiment			2nd Experiment			3rd Experiment		
	Decrease S. P.	Increase R. S.	Increase Viscosity	Decrease S. P.	Increase R. S.	Increase Viscosity	Decrease S. P.	Increase R. S.	Increase Viscosity
B. mes.....	0.30	0.01	—	0.20	—	—	0.05	—	—
B. liodermos.....	0.30	0.06	—	—	—	—	0.10	—	—
B. mes. vulgatus...	4.40	4.08	0.43	9.10	7.54	1.51	8.60	7.54	1.77
B. mes. ruber.....	1.20	0.93	0.26	6.50	6.89	0.97	6.50	6.55	2.45
Cult. X Bouillon...	8.80	4.61	1.96	8.40	11.00	1.95	8.80	7.69	1.83
Cult. X. S. S.....	8.50	3.18	1.93	9.05	10.80	2.27	8.40	7.40	1.83

It will be noted that only B. mes. ruber and B. mes. vulgatus acquired any sucrose destroying power, and the full power was recorded after a second transfer.

A careful examination of the summarized data of the three tables shows that only *B. vulgatus* and *B. mes. ruber* acquired an increased ability to destroy sucrose in a ten per cent sucrose solution. The increase in loss of sucrose in the second experiment over the first was very striking, particularly in the case of *B. mes. ruber*. This culture destroyed more than five times as much sucrose after the first acclimatization period as it did in the first experiment. In the case of *B. vulgatus* the increase was less striking, although very pronounced. It is interesting to note that the complete sucrose destroying ability seemed to have been acquired during the first acclimatization period, as no further

increase was noted in the third inoculation. Contrary to what might have been expected, the cultivation of the species of sugar bacteria in sucrose free bouillon did not tend to decrease its sucrose destroying power in ten per cent sucrose solution. From these experiments it appears evident that the cultures of potato bacteria when unacclimatized to sugar solutions do not possess as pronounced sucrose destroying ability as when grown for successive generations in contact with sucrose. This fact alone would tend to explain the great variation in gum fermenting power of cultures of sugar species isolated from different sugars. The question that arises in the light of the data shown in the preceding tables, is whether or not cultures of the potato species which have been acclimatized to ten per cent sucrose solution would be able to induce as relatively great a deterioration of higher density solutions.

TABLE IV.

Part II.

Table showing the action upon 34 Brix S. S. of Potato Bacteria Acclimatized to 10% S. S. and a Comparison with Sugar Species.

Cult.	Brix	Sucrose		Reducing Sugar	Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase in R. S.
		S. P.	S. C.				
Control.....	34.70	32.90	32.56	—	0.40	—	—
B. vulgatus.....	35.65	5.60	—	27.02	1.00	27.30	27.02
B. mes. ruber.....	34.85	6.40	—	25.00	1.20	26.50	25.00
Cult. X. Bouillon....	35.15	13.00	—	22.72	0.90	19.90	22.72
Cult. X. 10% S. S...	35.15	4.00	—	27.76	0.80	28.90	27.76

Having acquired a sucrose destroying power from having been acclimatized to 10% S. S., B mes. vulgatus and B. mes. ruber were as active in destroying S. S. as the cultures obtained from sugar

The data shown in the preceding table answer this question conclusively. The acclimatized cultures of the potato species of bacteria show as striking a sucrose destroying power upon a 34 Brix sucrose solution as upon a ten per cent solution. It is also interesting to note that the culture of the sugar species, which was grown in bouillon, showed a considerably lower sucrose destroying power than the same culture cultivated in a sucrose solution.

In connection with these experiments upon the acquired ability on the part of species of bacteria to ferment sucrose solu-

tions, it is interesting to note the observations of Kohn.[1] In his investigations upon water bacteria he found that species of those bacteria can be acclimatized to fairly high concentrations of sugar. The reversal of the process, i. e., the acclimatization to dilute solutions, take place much more rapidly than the former. He found that the limit of concentration for all of the species seemed to have been entirely governed by the osmotic pressure of the substance. Of more direct relation to the discussion of our data on acclimatization to sugar solution is the work of Landowsky,[2] who found that in plates to which transfers were made from a 30 per cent sodium chlorid bouillon, inoculated from garden soil, only *Bac. mesentericus* colonies developed. The result of that experiment, however, only indicated a resistance of the spores of *Bac. mesentericus* to high density salt solutions, for even in 25 per cent sodium chlorid bouillon that species would not develop. The resistance of the species of potato bacteria to the plasmolyzing effects of high density solution is no doubt due to the fact that they belong, as Fischer[3] has pointed out, to the group of bacteria which have a completely permeable cell membrane.

Whether or not the spores of the sugar species of bacteria are capable of developing in solutions of as high densities as those in which the vegetative cells of the species develop is a question of great practical importance as well as of scientific interest. Since most of the bacterial infection of sugars must result from the development of spores, it is of more than passing importance to determine if the maximum density in which spores can develop differs materially from the maximum for the vegetative cells. To determine whether such a variation exists between the spores and vegetative cells of the sugar species, the following experiment was conducted: A series of nutrient sucrose solutions was prepared, varying in density from 16 to 60 Brix. These solutions were then tubed and sterilized by the intermittent method of sterilization. They were then inoculated from a 24-hour growth of all the cultures of sugar species, as well as from spore preparations of the cultures. The spore preparations were made

[1]Weitere Beobachtungen Saccharophobe Bakterien cent. f. Bakt. Abt. II. XVII, 446.
[2]Ueber das Wachstum von Bakterien in Salzlosungen von Hoher Koncentration Archiv. f. Hygiene, Bd. XLIV, 1904, p. 47.
[3]Vorlesungen uber Bacterien.

by drying smears of old agar culture upon cover glasses, at a temperature of 125° for thirty minutes. The spore preparations were several months old and in an absolutely dry condition. It was scarcely possible, therefore, that they could have contained any living vegetative cells. The spores were introduced into the sterile tubes by rubbing a sterile platinum loop over the smeared surface of the cover glass, and then gently thrusting it into the tube.

TABLE V.

Experiment to Determine Limits of Density for the Development of Bacteria Cultures obtained from Sugar.

Culture	Nature of Inoculation	A		B		C		D		E		F		G		H	
		I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.
		16.6	18.20	26.15	—	30.38	31.24	30.10	36.40	40.03	40.54	42.24	43.6	44.95	46.14	55.37	55.44
		Effect Inoc.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.
I	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	48 hrs.	+	72 hrs.	+	96 hrs.		
	Spores.....	+	48 hrs.	+	60 hrs.	+	72 hrs.	+	72 hrs.	+	72 hrs.	+	96 hrs.	+	6½ days		
II	Veg. Cult....	+	12 hrs.	+	12 hrs.	+	12 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	5½ days		
	Spores.....	+	36 hrs.	+	36 hrs.	+	36 hrs.	+	84 hrs.	+	96 hrs.	+	5½ days	+	6 days		
Mixed Culture Sugar	Veg. Cult....	+	12 hrs.	+	12 hrs.	+	12 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	6 days		
IV	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	60 hrs.	+	60 hrs.	+	—	+	84 hrs.	+	96 hrs.		
	Spores.....	+	24 hrs.	+	60 hrs.	+	60 hrs.	+	60 hrs.	+	84 hrs.	+	84 hrs.	+	96 hrs.		
		16.73	16.87	26.75	26.77	30.45	30.62	35.65	35.61	40.45	40.70	42.83	42.90	45.12	45.31	55.22	55.44
VI	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	48 hrs.	+	72 hrs.	+	72 hrs.	+	84 hrs.	+	6 days		
	Spores.....	+	36 hrs.	+	48 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	96 hrs.	+	6 days		
VII	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	48 hrs.	+	72 hrs.	+	84 hrs.	+	5 days		
	Spores.....	+	48 hrs.	+	36 hrs.	+	48 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	6 days		
VIII	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	24 hrs.	+	48 hrs.	+	72 hrs.	+	84 hrs.	+	5 days		
	Spores.....	+	48 hrs.	+	48 hrs.	+	48 hrs.	+	96 hrs.	—	—	—	—	—	—	—	—
IX	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	24 hrs.	+	48 hrs.	+	72 hrs.	+	84 hrs.	+	5 days		
	Spores.....	+	48 hrs.	+	48 hrs.	+	48 hrs.	+	72 hrs.	+	96 hrs.	—	—	—	—		

I. B.—Initial Brix.
F. B.—Final Brix.
E. I.—Effect of Inoculation.

T. R. D.—Time required to develop—hours.
+—Growth.
— —No. growth.

TABLE V.---Continued.

Experiments to Determine the Limits of Density for the Development of Bacteria Isolated from Sugars.

Culture	Nature of Inoculant	B		C		D		E		F		G	
		Initial Brix	Final Brix	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.
		26.99	26.91	30.33	31.45	35.77	36.73	40.63	42.47	43.40	44.06	45.65	46.88
		E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.
XI	Veg. Cult.	+	12 hrs.	+	60 hrs.	+	60 hrs.	+	84 hrs.	+	5 days		
	Spores	+	72 hrs.	+	72 hrs.	+	72 hrs.	+	84 hrs.				
XII	Veg. Cult.	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	60 hrs.	+	60 hrs.	+	72 hrs.
	Spores	+	36 hrs.	+	36 hrs.	+	48 hrs.	+	60 hrs.	+	84 hrs.		
XIII	Veg. Cult.	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	60 hrs.	+	72 hrs.	+	72 hrs.
	Spores	+	36 hrs.	+	48 hrs.	+	84 hrs.	+	84 hrs.				
XIV	Veg. Cult.	+	12 hrs.	+	24 hrs.	+	36 hrs.	+	60 hrs.	+	72 hrs.	+	72 hrs.
	Spores	+	24 hrs.	+	36 hrs.	+	48 hrs.	+	60 hrs.	+		+	
XVI	Veg. Cult.	+	24 hrs.	+	36 hrs.	+	36 hrs.	+	48 hrs.	+	60 hrs.	+	6 days
	Spores	+	36 hrs.	+	60 hrs.	+	72 hrs.	+	96 hrs.	+	6 days	+	7 days
XVII	Veg. Cult.	+	24 hrs.	+	36 hrs.	+	36 hrs.	+	60 hrs.	+	60 hrs.	+	6 days
	Spores	+	36 hrs.										

I. B.—Initial Brix.
F. B.—Final Brix.
E. I.—Effect of Inoculation.
T. R. D.—Time required to develop.
+—Growth
——No Growth.

A study of the preceding table shows that the time required for any development to be observed in the tubes inoculated from the spore preparations was usually longer than in the tubes inoculated from the 24-hour cultures. In the lower series the difference in the time required for the development of spores and vegetative cells tends to remain constant throughout several of the various solutions. At the other extreme of the series the difference decreases, which is probably explained by the fact that in Series G the density was nearing the limit for the development of the vegetative cells as well as for the spores. The variation in the time of development of spores as well as vegetative cells does not increase directly with the density of the solution. There also appears to be a decided variation between the different cultures. An interesting fact in connection with this experiment is the application of the results to the principle of intermittent sterilization. It is obvious that the three successive day methods of sterilization could not be effectively used in the sterilization of high density sugar solution, if the solution carried as a part of its infection the spores of the sugar species of bacteria. Hence the three-day method of sterilization is still less applicable to the sterilization of sugars, that is, if it be *designed* to *destroy these* spores.

From the data in the tables under discussion it appears that the limit of density for all of the species of bacteria in sugars lies between 46 and 55 Brix. This is lower than in other experiments, where in certain instances growth was observed in solutions of slightly under 60 Brix sucrose solution. In the present experiments, however, some inversion had taken place in the sucrose solution, due to the successive heating of the tubes. It is quite likely, therefore, that the osmotic power of the solution had been sufficiently raised to depress the maximum density from the limit at which growth could have taken place in a pure sucrose nutrient solution.

TOLERANCE OF ACID AND ALKALI BY THE SPECIES OF SUGAR BACTERIA.

Since sugars vary considerably in acidity it is of importance to know the effect of the reaction of the medium upon the growth

of these bacteria. For this purpose an experiment was carried on upon a 10% sucrose solution, to which varying amounts of acid and alkali were added. The media were titrated before inoculation and again at the end of the experiment.

Toleracen of Acid by Cultures of Bacteria.

TABLE VI.

7/31/15

Culture	ACID – H_2SO_4											
	I		II		III		IV		V		VI	
	10 cc N/1-100 Titration		6 cc N/1-100 Titration		4-100 Titration		2.5-100 Titration		1-100 Titration		0.4-100 Titration	
	Before Sterilization	After End of 3 Days	Before Sterilization	At End of Exp...	Before Sterilization	At End of Exp.	Before Sterilization	At End of Exp.	Before Sterilization	At End of Exp.	Before Sterilization	At End of Exp.
Culture	8.8	5.6	5.6	5.6	3.7	4.0	2.1	2.0	1.2	1.3	0.6	0.6
I	–		–		–		–		–		+	
II	–		–		–		–		–		+	
III	–		–		–		–		–		+	
IV	–		–		–		–		–		+	
V	–		–		–		–		–		+	
VI	–		–		–		–		–		+	
VII	–		–		–		–		–		+	
VIII	–		–		–		–		–		+	
IX	–		–		–		–		–		+	
X	–		–		–		–		–		+	
XI	–		–		–		–		–		+	
XII	–		–		–		–		–		+	
XIII	–		–		–		–		–		+	
XIV	–		–		–		–		–		+	
XV	–		–		–		–		+		+	
XVI	–		–		–		–		–		+	
XVII	–		–		–		–		–		+	
XVIII	–		–		–		–		–		+	
B. vulgatus	–		–		–		–		–		+	
B mesentericus	–		–		–		–		–		+	

TABLE VII.

Tolerance of Acid by Cultures of Bacteria.

Culture	ACETIC ACID											
	I		II		III		IV		V		VI	
	Titration		Titration		Titration		Titration		Titration		Titration	
	Before Sterilization	After Sterilization	Before Sterilization	After Sterilization	Before Sterilization	After Sterilization	Before Sterilization	After Sterilization	Before Sterilization	After Sterilization	Before Sterilization	After Sterilization
Culture	8.8 cc	8.8 cc	5.6	5.6	3.9	3.4	2.0	1.8	1.3	1.3	0.8	0.8
I	–		–		–		–		–		+	
II	–		–		–		–		–		+	
III	–		–		–		–		–		+	
IV	–		–		–		–		–		+	
V	–		–		–		–		–		+	
VI	–		–		–		–		–		+	
VII	–		–		–		–		–		+	
VIII	–		–		–		–		–		+	
IX	–		–		–		–		–		+	
X	–		–		–		–		–		+	
XI	–		–		–		–		–		+	
XII	–		–		–		–		–		+	
XIII	–		–		–		–		–		+	
XIV	–		–		–		–		+		+	
XV	–		–		–		–		–		+	
XVI	–		–		–		–		–		+	
XVII	–		–		–		–		–		+	
XVIII	–		–		–		–		–		+	
B. vulgate	–		–		–		–		–		+	
B. mes	–		–		–		–		–		+	

Tolerance of Alkalinity by Cultures of Bacteria.

TABLE VIII.

7/3/15.

Culture	ALKALIES. NaOH.									
	I		II		III		IV		V	
	10-100 Titration		6-100 Titration		4-100 Titration		2.5-100 Titration		1-100 Titration	
	Before Sterilizatn	After Sterilizatn	Before	After	Before	After	Before	After	Before	After
Culture	–	5.8	4.6	2.40	3.2	1.1	1.5	0.6	0.7	0.3
I	–		–		–		–		+	
II	–		–		–		–		+	
III	–		–		–		+		+	
IV	–		–		–		–		+	
V	–		–		–		–		+	
VI	–		–		–		–		+	
VII	–		–		–		–		+	
VIII	–		–		–		–		+	
IX	–		–		–		–		+	
X	–		–		–		–		+	
XI	–		–		–		–		+	
XII	–		–		–		–		+	
XIII	–		–		–		–		+	
XIV	–		–		–		+		+	
XV	–		–		–		–		+	
XVI	–		–		–		–		+	
XVII	–		–		–		–		+	
XVIII	–		–		–		–		+	
B. vulgate	–		–		–		–		+	
B. mes.	–		–		–		–		+	

Note low tolerance of cultures towards alkalinity.

The following tables give the data from the experiment. The degree of acidity or alkalinity is expressed in terms of cc of N/10 Na(OH) or H_2SO_4 required to neutralize 10 cc of the solution. It will be noted that the limit of acidity for the majority of cultures was about 0.6 to .8 cc. There did not appear to be any appreciable difference in the degree of tolerance to acetic and to sulphuric acid. The tolerance of the cultures to both acid and alkali seems remarkably low. Lehmann and Newmann[3] report an excellent development of both *Bac. vulgatus* and *Bac. mesentericus* in media, showing a reaction of+2 and —1, Fuller's scale. Edson and Carpenter[4] report excellent growth of the micro-organisms isolated from maple sap in media having an optimum reaction of +10. It was to be expected that the tolerance of acids and alkalies in a sugar solution would be different from the result obtained in bouillon, but such a low tolerance to these substances was not anticipated.

THE RELATIVE SUCROSE DESTROYING POWER OF THE VARIOUS CULTURES OF BACTERIA OBTAINED FROM SUGARS.

The fact that the various cultures of bacteria obtained from sugars vary greatly in their ability to destroy sucrose has been pointed out by all who have investigated the subject of sugar deterioration. Lewton Brain and Noel Deerr[1] determined this variation on 10% sucrose solution. Greig Smith[2] also referred to this variation in his descriptions of the bacterial species in sugars. In order to determine this variation in the sucrose destroying power of the cultures of bacteria under investigation, the author decided to test it upon sucrose solutions of higher density than those previously used. With this in view, a nutrient sucrose solution was made of about 52 Brix, using the nutrient salts in the proportions prescribed in Smith's formula for 10% sucrose solution.

[1]Lehmann and Newmann Medical Atlas.
[2]Micro-organisms of Maple Sap, Bul. 167, Vermont Eqp. Station.
[3]Loc. cit.
[4]Loc. cit.

The following table shows the results of a 30-day incubation period of the inoculated flasks:

TABLE IX.

Experiment on the Comparative Deteriorative Powers of Different Cultures of Bacteria upon 50% S. S.

Inoculation	Sucrose		Reducing Sugar	% Total Solids	Period of Incubation	Decrease Sucrose	Increase R. S.
	S. P.	S. C.					
Control	51.50	52.44	0.03	52.81	30 days	—	—
Cult. I	26.40	—	18.80	53.76	"	25.10	18.77
" II	25.40	—	19.20	53.76	"	26.10	19.17
" III	51.90	52.40	0.01	53.33	"	0.04	—
" IV	26.80	—	19.05	53.80	"	24.70	19.02
" V	15.20	—	25.60	53.55	"	36.30	25.57
" VI	12.00	—	29.40	53.37	"	39.50	29.47
" VII	12.00	—	30.90	53.22	"	39.50	30.87
" VIII	14.20	—	27.80	53.22	"	37.30	27.77
" IX	17.00	—	26.90	52.97	"	34.50	26.87
" X	15.20	—	27.50	53.30	"	36.30	27.47
" XI	9.60	—	30.20	53.53	"	41.90	30.17
" XII	27.80	—	17.50	52.80	"	23.70	17.47
" XIII	10.60	—	29.40	53.05	"	40.90	29.37
" XIV	11.80	—	27.80	53.30	"	39.70	27.77
" XV	52.26	52.73	0.80	53.61	"	—	0.77
" XVI	51.22	51.95	0.91	53.13	"	0.28	0.88
" XVII	52.00	52.40	0.02	53.13	"	0.04	—
" XVIII	52.52	53.02	0.02	53.77	"	—	—
Vulgatus	42.64	—	7.90	53.29	"	8.86	7.87

NOTE: Strong deteriorative power of Cult. XI.
Low deteriorative power of Cult. XVI which has high gum-forming power on 10% S. S.
Non gum-forming species no action on 50% S. S.

It will be noted that there is a great variation in the sucrose destroying power of the various cultures. This variation covers a range of 41 in the case of Culture XI to 24 in the case of Culture IV. Some of the cultures, which had been found to cause a rapid deterioration of 10% sucrose solution, were inactive in the above experiment. An unacclimatized culture of *Bac. vulgatus* was used in the experiment, which accounts for the comparatively small destruction of sucrose. The inert cultures of bacteria, III, XV, XVIII, were used in conjunction with the other cultures, and, as was to be expected, they induced no change in the composition of the solution. We have already referred to the fact that much of this variation in the sucrose destroying power of bacterial cultures isolated from sugars may be due to the respective stages of acclimatization which the different cultures may have reached. In the above experiment, however, all of the cultures, with the exception of *Bac. vulgatus*, which was grown in plain agar, had been to an equal degree acclimatized to sugar solution.

The difference in the sucrose destroying power of the various cultures of bacteria occurring in sugars has been offered as an explanation of the different behavior in storage of sugars of the same type. It has not been our experience that sugars vary to any great extent in the complexity of their bacterial infection. The difference in the bacterial infection of various types of sugar seems to be a difference of degree and not of kind. A Cuban or Porto Rican 96-test sugar is, so far as we have been able to observe, likely to carry the same species of bacteria as are found in sugars from Hawaii or Louisiana. The bacterial infection of all sugars originates from the same source, *i. e.*, from the potato group of bacteria. It is very likely true that the original bacterial infection of the majority of sugars is composed of both acclimatized and unacclimatized cultures. The former class comes from the dust and old sugar products in the factory. The latter class is composed of the spores of the bacteria in the raw juice, which persist throughout the milling process.

THE ACTION OF PURE CULTURES OF SUGAR BACTERIA UPON STERILIZED SUGARS.

In the previous experiments upon the limits of density within which the cultures of sugar bacteria can destroy sucrose, we found that it was comparatively low in all cases. No growth, in fact, was obtained in any case in the 55 Brix solution. This limitation of density seems to preclude the possibility of these cultures causing the deterioration of sugars, except in rare instances where the molasses film is greatly diluted by the absorption of moisture. The following table gives the results of a one-month incubation period of sugars inoculated from pure cultures of sugar bacteria. The sugar was sterilized for one hour at 212°, then allowed to absorb moisture under a bell jar prior to inoculation.

TABLE X.

PART II A.

Experiment on Inoculation of Sugars.
Sugar from Sugar House.
Sterlized at 212°

(Incculation 1 month). (Inoculation from plain Agar Streak).

Culture	% Moist.	R. S.	Sucrose		Dry Basis		No. Micro. per Gram.	Factor
			S. P.	S. C.	S. P.	S. C.		
.....	3.11	0.84	94.6	95.75	97.63	98.82	5,333	.575
II.....	3.00	0.87	94.6	95.75	97.52	98.71	32,000	.555
III.....	3.11	0.83	94.6	95.75	97.63	98.82	120,900	.575
IV.....	2.91	0.80	94.8	96.12	97.64	99.00	89,300	.559
V.....	3.00	0.84	95.2	96.26	98.14	99.23	1,800	.624
VI.....	3.18	0.86	95.4	96.58	98.53	99.75	39,030	.691
VII.....	2.88	0.82	94.10	95.36	96.88	98.19	71,500	.488
VIII.....	3.00	0.81	94.40	95.60	97.32	98.55	130,500	.535
IX.....	2.91	0.86	95.30	96.24	98.15	99.12	28,200	.619
X.....	3.18	0.87	95.00	96.18	98.12	99.34	2,376	.636
XI.....	2.61	0.83	95.40	96.48	97.95	99.06	35,000	.566
XII.....	2.89	0.83	95.90	96.88	98.75	99.76	10,300	.704
XIII.....	2.91	0.86	95.00	95.83	97.84	98.71	5,500	.582
XIV.....	2.96	0.90	95.10	96.07	98.00	99.00	64,000	.604
XV.....	2.64	0.82	95.50	96.21	98.09	98.82	1,700	.586
Control...	4.06	0.86	94.00	94.72	97.97	98.73	.....	.676

In the above table it will be noted that although the ratio of solids non sucrose to moisture was very low, giving a factor, which in some cases was as high as .704, yet no appreciable deterioration took place.

Table XI shows the results of another experiment upon the inoculation of sterilized sugar with Cultures I and II sugar bacteria, and also with Culture I torula, the latter also isolated from sugar. The sugar used in this experiment was of the washed 96-test type. It was sterilized at 212° for one hour upon intermittent days, allowing for two-three days' interval between the sterilization periods. After sterilization, the sugar was allowed to absorb moisture and inoculated as in the previous experiment.

TABLE XI.

Inoculation Experiment. Sugar from Station Sugar House. Sterilized and Inoculated in 1 Liter Flasks. 7/31/15.

Inoculation	Sucrose		Reducing Sugar	Moist.	Period of Incubation	Dry Basis		Decrease in Sucrose	Increase in R. S.
	S. P.	S. C.				S. P.	S C.		
Original Sample...	97.20	98.88	0.80	0.90	30 days	98.08	99.77	—	—
Control.....	88.80	90.32	1.49	7.89	"	96.40	98.06	—	—
Cult. I Yeast.....	91.70	93.10	0.32	5.95	"	97.50	98.99	+0.93	−1.17
Cult. I Bact.....	78.90	82.31	10.00	6.37	"	84.27	87.91	−10.15	+8.51
Cult. II Bact.....	91.50	93.04	1.18	5.47	"	96.79	98.41	+0.35	−0.31

Sterilization in autoclave at 212° F. for one hour on 3 days at 2-3 day intervals. Inoculated with platinum loop of a 24-hour agar streak culture.

NOTE: Rapid deterioration of sample inoculated with Bact. Cult. and decrease in reducing sugar in sample inoculated with yeast.

The results of this experiment show a pronounced ability on the part of Culture I bacteria to destroy sucrose. The flask inoculated with Culture II bacteria did not deteriorate, which is probably explained by the fact that it contained nearly one per cent less moisture. We might well inquire why the culture of bacteria caused a deterioration of the sugar in this case and none in the previous experiment. We find the ratio of solids non sucrose to moisture approximately the same in both cases. The explanation of this difference is to be found in the difference in the types of sugars used in the two instances. In the second experiment we used a washed 96-test sugar, a type which our experience has shown to be more susceptible to deterioration than is unwashed sugar of the same grade. This fact was noted in Part I of this publication. The results of these experiments show the characteristic glucose destroying power of the torula, which was also discussed in Part I. A further discussion of it will be reserved for the next chapter.

The effect of the inoculation of sugars with bacteria will be treated more fully in a subsequent chapter of this publication. It is nevertheless opportune to discuss here in connection with the characteristics of these species the role played by them in sugar deterioration. *A critical review of the data now extant on this subject shows that no positive results have been obtained from the inoculation of sterile sugars with pure cultures of bacteria, except in cases where the sugar used would be considered abnormal as regards its moisture content.* A review of the literature on the subject shows the following general results:

Greig Smith[1] does not report any experiments upon the inoculation of sterile sugar. Dodson[2] reports positive results from his experiments, but the incubation period was two and a half years' duration, and the moisture content of the sugar is, unfortunately, not reported. Brain and Deerr[3] obtained positive results from their bacterial inoculations, but the moisture content of their sugars varied from a minimum of approximately 2.2 to a maximum of over 4 per cent. As compared with a normal Hawaiian 96-test sugar, it is apparent that their inoculated

[1]Loc. cit.
[2]La. Bul. No. 75.
[3]Loc. cit.

sugars carried an excessive amount of moisture. Owen[1] in a previous publication reported results from inoculations. Although the moisture content of the 96-test sugars used was in most cases between 2 and 3 per cent, the deterioration during an incubation period of about three months was very small and entirely negligible in many instances.

If we take the limitation of density into account in explaining the spheres of activity of bacteria in different types of sugar, then we must also apply the same limitation in explaining the limit to which this action can extend. If the molasses film surrounding the sugar crystal must be comparatively dilute to enable the bacteria to destroy sucrose, then the film must contain a correspondingly small amount of sucrose. Nor is it probable that the conditions resulting from the destruction of this sucrose would be conducive to a further dissolving of the sugar crystal. On the contrary, since the dissolved sucrose is transformed by the bacteria into gum and reducing sugar, the solvent action of the film moisture would more likely be decreased rather than increased. This decreased solubility of the sugar crystal in the surrounding film resulting from the formation of solids non sucrose by bacterial action appears even more probable in the light of another theory. Thus Browne[2] mentions as a possibility that these solids non sucrose in the molasses film may seal off the sugar crystal from the film and thus prevent the latter from taking up more sucrose.

SUMMARY OF PART II, A.

(1) The gum forming bacteria which occur in sugars may be regarded as derived types of the potato group of bacteria. The majority of the cultures investigated seem more closely related in morphological and physiological characteristics to *Bac. vulgatus* than to the other members of the group.

(2) *Bac. vulgatus* and *Bac. mes. ruber* when isolated from sources other than sugar products have a comparatively low sucrose destroying power. This power can be greatly increased by cultivating these species for successive generations in a nutrient sucrose solution. Having acquired the power, on a

[1]La. Bul. No. 125.
[2]La. Planter, Vol. LIV, No. 18, 1907, p. 28.

10% sucrose solution, they seem capable of exercising it to a corresponding extent upon a sucrose solution of 34 Brix. This acquired power does not appear to be easily lost when the cultures are cultivated on sugar free media.

(3) The limit of sucrose concentration in which the cultures seem capable of inducing any appreciable action is between 46 and 55 Brix. While there is a difference in time required by the spores and vegetative cells of each culture to develop in sucrose solutions, this difference is not proportionate to the density of the solution.

(4) The cultures of bacteria from sugars have a low tolerance of acid and alkali when grown in sucrose solutions.

(5) The comparatively low maximum density in which these bacteria are capable of developing, suggests that their deteriorative action upon sugars is largely confined to fairly moist sugars, in which the molasses films have been much diluted by the absorption, or addition of moisture.

PART II B.

TORULA.

While investigating[1] the comparative value of various culture media for the quantitative bacteriological analysis of sugars, it was found that the use of the ordinary 10% sucrose agar prevented a very important group of micro-organisms in sugars from developing on the plates. The use of sucrose media of higher density resulted in the isolation of a fairly large number of these cultures. It showed, furthermore, that most sugars contain a larger number of this group of micro-organisms than of bacteria. The occurrence of torula in sugar products has been noted by many investigators, but their action in that environment has not up to this time been closely studied. Jörgensen[2] reports the presence of these forms in sugar factories, and even in the finished sugar. Both Laxa[3] and Schone[4] reported the predominance of torula in plates made from beet juice taken from the diffusion batteries at 30°C.

[1]Bulletin 146, La. Exp. Station.
[2]Micro-organisms and Fermentation, p. 385.
[3]Vereins Zeitschr 1901, Bd. 51.
[4]Bohm Zeitschr 1901, Bd. 26.

Torula have also been observed in condensed milk and are regarded[1] by some as the chief cause of the fermentation which sometimes takes place in these products, causing considerable losses to the manufacturers. The association of these forms with diseased beers is well known. Edwards[2] has made an extensive study of the role played by torula in the production of flavors in cheese. In the cane sugar industry their action is not only the occasion of concern in the deterioration of sugars, but also in the fermentation of table syrups.

For the most extensive study of torula now extant, we are indebted to Will[3]. His investigations of the torula occurring in breweries are very comprehensive, giving a complete morphological description of the species during their cultivation on liquid and solid media. He also records the action of the species with the different sugars, their production of acids and their behavior in competition with culture yeast, etc.

The isolation of torula from sugars was accomplished by the inoculation of a 35 Brix agar with small portions of sugar samples. Their growth in this medium is somewhat slow, but the colonies attain macroscopic dimensions in from two to three days. The sugar agar colonies of the different cultures show considerable distinctiveness. Seventeen distinctive cultures were obtained in the course of the investigation.

The following are the principal morphological and cultural characteristics of the cultures:

Culture I.

Cells spherical or slightly elliptical in shape; weakly stained with Grams iodine solution; cells varying in diameter from 3-7M. No spores observed.

On potatoes the growth is grayish white; and dry, and confined to within one-eighth of an inch of line of smear.

On 50% sucrose agar slants, the growth is brown and smooth.

On molasses agar the growth is smooth raised, and very dark in color. Fifty per cent sucrose gelatin not liquefied in two weeks.

[1]Lafar Handbuch der Technischen-Mykologie, Bd. II, 292.
[2]Fruity, or sweet flavor in cheddar cheese. Cent fur Bakt. Abt. II 1913, Bd. 39
[3]Cent fur Bakt. Abt. II 1907, 1908, Bd. 39, 450.

Culture II.

In shape and size similar to Culture I; spherical cells vary from 3.4-6.2M in diameter. No spores observed; not stained with iodine. On potato slants a more abundant growth than with Culture I. The growth is most raised on edges, and of a creamy color. On 50% sucrose agar slants the growth is smooth, and white. On molasses agar as in Culture I.

Culture III.

Cells shorter and smaller in diameter than the above culture. No spores observed. Cells not stained with iodine solution. On potato slants the growth is smooth, flat and spreading. On 50% sucrose agar the growth is smooth and flat. On molasses agar slants the growth is smooth and flat.

Culture IV.

Small round cells, varying in diameter from 3-4M; no spores observed.

Culture VI.

Cells round and elliptical, varying in size from 4 to 9M. Weakly stained with iodine solution. No spores observed.

On potato slant the growth is moist white and glistening, and more luxuriant than the previous culture.

On agar slants like culture previously described.

On molasses agar slants, the growth is smooth and very dark.

Culture VIII.

Very small cells, from 3-5M diameter. On potato the growth is white, flat and spreading. No spores observed.

On 50% sucrose agar the growth is smooth raised, and light brown in color.

On molasses agar slants the growth is smooth and raised, and very luxuriant.

Culture IX.

Small cells as in No. VIII, but more elliptical. Length 5M. No spores observed.

On potato the growth is very feeble, and barely discernible after two weeks' incubation.

On 50% sucrose agar the growth is raised and luxuriant.

On molasses agar slant the growth is filamentous, like Culture IV, except that it is much darker in color.

Culture X.

Small cells resembling the lemon-shaped cells of *Saccharomyces apiculatus*. Cells weakly stained with iodine. On sucrose agar the growth is slow. No spores observed.

On potato slants the growth is more abundant. The culture forms a yellowish spreading growth on the entire upper surface. On molasses agar slants the growth is similar to Culture IX.

Culture XI.

In both shape and size of cells this culture varies from the ones previously described. The cells are very large, and very unsymmetrical in shape. No spores observed.

On potato slant the growth is moist and raised, and grayish white in color.

On molasses agar slants the growth is similar to Culture IX.

Culture XII.

Small elliptical cells as in No. X. On potato slants the growth is white and dry. The cells are more deeply stained with iodine than was true of No. X.

On molasses agar the growth is smooth and very black.

Culture XIII.

In size and shape like Culture XII. Cells very weakly stained with iodine. Colonies on sucrose agar very small and round. On potato slants the growth is very feeble.

On molasses agar the growth is very black, but filamentous like Culture IX.

Culture XIV.

Cells larger and more deeply stained with iodine than Culture XIII.

On potato the culture resembles Culture XIII. On sucrose agar the growth is much more luxuriant than in the preceding culture.

On molasses agar the growth is very similar to Culture XIII.

Culture XV.

Very small spherical cells, giving a very pronounced glycogen reaction with iodine.

On potato slants the growth is very luxuriant; gray growth; edges of growth irregular.

On sucrose agar the colonies are very small, and only slightly raised.

On molasses agar slants, smooth brown growth.

Culture XVI.

Cells darkly stained as above. More elliptical in shape, and larger in size.

On potato slants there is a very luxuriant dry growth.

The colonies on sucrose agar are larger and more irregular than from previous cultures.

Culture XVII.

The cells are very long and narrow. Stain very deeply with iodine. Cells are grouped in characteristic bunches. Very distinctive in cell grouping.

On potato slants, the growth is very feeble and forms an almost transparent veil over the surface of the potato.

On molasses agar the growth is very dark and smooth.

Schizosaccharomyces in Sugars.

In the course of our investigations of the yeastlike microorganisms in sugars, we encountered two cultures of spore-forming fission yeast, both of which seem closely related to *Saccharomyces octosporus* (Beijerinck)[1].

[1]Cent. fur Bakt. XVI, 1894. For a complete detailed description of this species the reade- is referred to Schionning's excellent work, a resume of which is given in Jorgensen's micro organisms and Fermentation, pp. 377-381.

Culture VII.

This culture resembles No. V, but the cells are not as much constricted in the center. Cultural features agree with those of No. V. This culture also forms endogenous spores, which are usually four in number.

Additional Features.

Most of Will's cultures of torula liquefied gelatine. Only a few of those found in sugars were capable of liquefying a 25% sucrose gelatine.

The cultures do not coagulate milk, although they produce various characteristic odors in it. This is in agreement with Will's observations, as is also the difference in the glycogen reaction of the various cultures. The question of whether the cultures do or do not form spores was tested on gypsum blocks, as well as on potato slants, both methods being well calculated to demonstrate the possession or the lack of this ability on the part of these micro-organisms.

The wide scope of this investigation of sugar deterioration rendered impossible a more detailed study of the morphological and physiological characteristics of this interesting group of micro-organisms. The author is free to confess that the data here presented on that subject leaves much to be desired so far as regards the finer and more delicate differentiating characteristics of the torula in cane sugars.

For the present, we must concern ourselves more with the action of these cultures upon sugar products than with the elaboration of more refined methods of differentiating the species.

The occurrence of Saccharomyces octosporous-like species in cane sugar is of especial interest in view of the fact that a patent for its use as a means of recovering sucrose from exhausted cane molasses was granted to Glashan[1]. For the same purpose Pellet and Pairault[2] believed they had isolated an even more suitable yeast.

[1]Lafar Handbuch der Technischen Mykologie, Bd. II, p. 500.
Bul. Assoc. Chim. de Sucr, et de dist, 1905, Bd. 23, s639.
[2]For a very clear explanation of the impracticability of this process, the reader should consult Brown's Handbook of Sugar Analysis, p. 651.

Culture V isolated from Cuban sugar (cells varying in shape from spherical to characteristic dumbbell forms). In size from 5-8M and 16M in length. The cells lengthen before fission, and again coalesce so as to form the elliptical dumbbell form.

This culture appeared to form only four spores, which is frequently the case with the *Saccharomyces octosporous.*

On potato the growth is moist, flat and white, spreading over the entire surface of the potato.

On molasses agar the growth is filamentous in form, and brown in color.

ACTION OF TORULA CULTURES UPON SUGAR SOLUTIONS.

In the following table is given the results of an experiment upon the action of the torula cultures upon a 50% sucrose solution, made according to Smith's formula. The period of incubation was one month.

TABLE I.

Experiment upon the Action of Yeast Cultures obtained from Sugars, upon 50% Sucrose Solution. (Smith's formula).
Incubation 1 month.
Inoculated from Agar tubes.

Culture	Total Solids Brix	R. S.	Sucrose	
			S. P.	S. C.
Control	51.40	.06	50.70	51.20
Yeast Culture I	51.68	.09	50.55	50.93
" II	51.23	.35	49.10	49.61
" III	50.73	.10	50.10	50.37
" IV	50.73	.35	49.60	49.90
" V	50.03	.28	47.90	49.18
" VI	47.75	8.33	36.20	35.95
" VII	51.15	.03	49.85	51.13
" VIII	51.15	.10	49.95	50.35
" IX	50.65	.08	49.55	49.90
" X	50.65	.41	49.40	49.57
" XI	51.68	.08	50.40	50.60
" XII	50.20	.40	48.05	Lost
" XIII	51.85	.39	48.85	49.39
" XIV	51.85	.33	48.75	48.62
" XV	46.22	1.56	39.75	40.61
" XVI	50.12	4.17	41.00	42.26
" XVII	52.43	.13	49.80	50.38

From the above table it will be seen that in most cases the destruction of sucrose by these cultures was very small. It was highest in Culture VI and Culture XV, but even then it was very weak as compared with that of the bacterial cultures from sugars, when grown in a solution of the same composition. It will be

noted that in most of the flasks the amount of invert sugar, at the end of the experiment, was little less than one-half of the amount of sucrose destroyed. In Will's investigation of the torula in breweries, he found that the different cultures varied greatly in their power to invert sucrose. Many lacked this property entirely, others possessed it in degrees varying from very weak to fairly strong. As regards the ability of his cultures to ferment dextrose and levulose, he found that in most cases both of these sugars were utilized to an equal degree. In some cases, however, dextrose was more readily attacked, and in others levulose. The degree of the selective action, however, was never very pronounced.

ACTION OF THE CULTURE IN 50% DEXTROSE AND SIMILAR SOLUTIONS.

TABLE II.

	Levulose	Dextrose
Control	50%	50%
Control	50	50
I Cult.	49.50	48.78
II "	49.02	47.39
III "	47.61	49.02
IV "	50.00	49.50
V "	48.07	49.02
VI "	50.00	47.39
VII "	48.78	48.78
VIII "	47.61	46.51
IX "	49.50	48.78
X "	48.78	48.07
XI "	47.17	49.02
XII "	49.02	47.61
XIII "	48.78	49.02
XIV "	47.50	47.61
XV "	48.54	48.54
XVI "	49.02	48.78
XVII "	48.78	48.54

LEVULOSE—DEXTROSE.

It will be noted from the above table that there seems to be no decided selective action of the cultures of torula for either of the two sugars. Both dextrose and levulose seemed to have been utilized with equal facility by each culture. The amount of fermentation was comparatively small in all cases.

ACTION OF TORULA CULTURES UPON DILUTED FINAL MOLASSES.

A final molasses was diluted to 40 Brix and 200 cc portions poured into flasks and sterilized by the intermittent method. The flasks were then inoculated from molasses agar cultures of the torula.

TABLE III.

Experiment On the Action of Yeasts Isolated from Sugars Upon Half Dilution Molasses.
One month's incubation period. Inoculated from agar tubes of half dilution molasses.

Cult.	Total Solids	Reducing Sugars	Sucrose Direct Polarization	Sucrose Clerget	Total Sugars Loss	
Control I	40.70	18.3	9.10	12.28	30.58	.....
" II	38.70	18 3	9.6	11.69	29.97	.....
Average Control	39.70	18.3	9.35	11.98	30.27	.....
Yeast Cult. I	34.30	19.0	5.6	3.81	7.46	.....
" II	33.75	18.0	6.0	4.39	3.88	.....
Cult. III	34.30	18.3	7.4	5.41	6.56	.....
" IV	34.85	20.2	7.4	5.07	5.00	.....
" V	34.74	20.2	9.4	4.13	6.12	.....
" VI	33.66	17.6	10.0	4.08	8.59	.....
" VII	36.91	18.3	7.6	4.51	2.41	.....
" VIII	35.81	20.2	7.3	3.94	6.13	.....
" IX	36.91	20.2	8.0	3.89	6.18	.....
" X	35.90	20.2	9.6	3.42	6.65	.....
" XI	35.35	20.2	8.4	3.18	6.89	.....
" XII	29.30	17.5	10.4	3.78	8.99	.....
" XIII	35.35	20.9	9.5	3.93	5.44	.....
" XIV	30.40	20.2	9.8	4.50	5.57	.....
" XV	34.45	17.2	11.0	4.10	8.97	.....
" XVI	34.45	21.3	8.0	3.56	5 41	.....
" XVII	35.00	19.6	4.2	3.67	7.00	.....
Bact. Cult. I	40.30	18.7	10.2	12.15	.....	Av. loss in Sucrose 6.30

The results of the experiment were interesting, since the amount of invert sugar in the inoculated flasks tended to remain constant in spite of the fact that some inversion had taken place. The increase in invert sugar in the inoculated flasks as compared with the control was never more than 2%, while the decrease in sucrose in these flasks was 7-8%. The experiment was repeated, with the results shown in the following table:

TABLE IV.

Half Dilution Molasses Inoculated with Yeast Cultures from Sugar Inoculated October. Analyzed November.

Sample	Inoculated Culture	Total Solids	Reducing Sugars	SUCROSE			Loss in Total Sugars
				S. P.	S. C.	Total Sugars	
1 Control	.....	38.70	12.6	11.0	13.69	26.29	.....
2	.....	.....	.....	.....	.....	.....	.....
3	Cult. I	32.92	9.5	2.74	0.61	10.11	14.81
4	" II	32.58	10.6	3.70	1.37	11.97	12.95
5	" III	33.93	11.5	3.34	4.57	16.07	8.85
6	" IV	32.80	14.8	2.22	3.04	17.84	7.08
7	" V	32.80	14.24	4.32	4.31	18.55	6.37
8	" VI	30.52	9.30	8.26	7.66	16.96	7.96
9	" VII	31.63	10.00	8.30	7.35	17.35	7.57
10	" VIII	32.32	10.40	8.20	7.62	18.02	6.90
11	" IX	33.80	14.10	8.00	8.32	22.42	2.50
12	" X	34.38	13.7	7.64	7.72	21.42	3.50
13	" XI	34.08	16.4	7.24	7.97	24.37	0.55
14	" XII	33.83	9.05	9.80	9.40	18.45	6.47
15	" XIII	33.90	12.00	14.16	12.45	24.45	0.47
16	" XIV	34.38	12.56	11.00	9.50	22.06	2.86
17	" XV	34.13	8.70	10.00	8.72	17.42	7.50
18	" XVI	35.28	12.40	12.7	11.18	23.58	1.34
19	" XVII Y. & B.	33.88	10.00	7.65	7.35	17.35	7.57
20	" XVII Y.	34.13	10.8	6.44	7.15	17.95	6.97
21	.....	.....	.....	.....	.....	.....	Av. Loss 6.22

In this experiment the loss in sucrose was higher in some cases than in the previous experiment. There was also a greater tendency for the invert sugar of the inoculated samples to vary from that of the control. This variation took the form of an increase in certain cases and a decrease in others. The limits of the variation seemed to be about 3% either way. In other words, it appeared that in some cases the torula cultures formed more invert sugar than they could ferment during the period of incubation, and in other cases, less. An average of all the inoculation results tends to show a preference on the part of the organisms to ferment only as much invert sugar as it formed from the inversion of the sucrose in the solution. An interesting point in connection with the above table is the fact that in many cases the single polarization of the inoculated samples was higher than the sucrose Clerget. This indicated a selective action of certain of the cultures upon levulose, which resulted in an accumulation of dextrose in the solution.

The fermentation of molasses and syrups by torula often results in the development of various esters. It is to the formation of such products that deteriorating sugars owe their characteristic aroma. The fruity odor of deteriorating sugars has been

mentioned by various writers. Thus Browne[1] calls attention to this fact as follows:

> *"The strong odor of esters, which is so perceptible upon entering a warehouse or the hold of a ship where raw sugar is stored, is produced by micro-organisms. This odor is sometimes regarded as an evidence of deterioration, but this is not necessarily the case. Sealed samples of raw sugars may develop an intense rum-like odor without showing the slightest loss in polarizaton."*

We will discontinue the further consideration of this group of micro-organisms, but will again advert to it in a subsequent review of the comparative action of the various groups of micro-organisms upon cane sugars and cane sugar products.

PART II C.

MOULDS.

As far as can be learned from the fragmentary literature on the subject of sugar deterioration, it appears that it was Shorey who first attributed such changes in the composition of sugars during storage to the activities of moulds. In a paper,[2] presented in 1898 at the May meeting of the Society of Chemical Industry, the author above referred to stated that it is *Penicillium glaucum,* and not bacteria, that are responsible for the deterioration of raw cane sugars. Shorey's work, while highly valuable, was not sufficiently complete, and it could not, therefore, have been expected to have resulted in a prompt and general acceptance of his theory. His theory was founded on the following observations:

(1) The presence of *Penicillium glaucum* in sugars.

(2) The deterioration of alkaline sugars and the ability of *Penicillium glaucum* to invert sucrose solutions of a similar degree of alkalinity.

(3) The development of *Penicillium glaucum* in solutions made from deteriorating sugars.

[1]Chemical Factors in the Deterioration of Raw Sugars.
[2]The Deterioration of Raw Cane Sugar in Transit or Storage. The Journal of the Society of Chemical Industry, June, 1898.

While these deductions might very probably have been the true explanation of one of the causes of sugar deterioration, yet the experimental data presented did not eliminate other causes, which might also have been operative. Shorey himself claimed for his deductions an application only to the special cases which had come under his observation.

The fact that subsequent investigators did not build upon the foundation which Shorey had laid was due to the following causes, viz.: (1) The predominance of bacteria developing upon plates made from sugars, which was due to the prevalent use of a medium especially favorable to that class of micro-organisms. (2) The incompleteness of Shorey's data.

Thus we find Dodson,[1] Greig Smith,[2] Deerr,[3] Norris,[4], Brain[5] and Owen[6] leaving moulds out of consideration as one of the causative agencies in sugar deterioration.

It is true that Deerr and Norris[7] admitted that in certain cases the numbers of bacteria in some of the samples of deteriorating sugar which came under their observation were too low to account for the chemical changes which occurred. They did not, however, attribute these changes to any special group of micro-organisms. In connection with the study of the deterioration of beet sugars, Schone[8] mentions the occurrence in these products of *Penicillium glaucum,* and also, but much less frequently, aspergillus and mucor species. He did not credit them, however, with any definite connection with the deterioration of sugars.

The secretion of invertase by species of the aspergillus, penicillium and other groups of fungi has long been known. As early as 1878 Gayon[9] found that aspergillus niger formed invert sugar when grown in a medium containing cane sugar. This discovery was confirmed by Duclaux[10] in 1883. The isolation of invertase from this species of mould was successfully carried out for the first time by Fernbach in 1890. The isolation of invertase from

[1]Loc. cit.
[2]Loc. cit.
[3]Loc. cit.
[4]Loc. cit.
[5]Loc. cit.
[6]Loc. cit.
[7]Loc. cit.
[8]Latar Handbuch der Technischen Mykologie Bd. II.
[9] [10]Soluble Ferments and Fermentation, p. 121. J. Reynolds Green.

Penicillium glaucum was effected by Bourquelot in 1886. The limits of density, however, in which these species are able to invert sucrose does not seem to have been thoroughly studied.

THE ISOLATION OF MOULDS FROM SUGARS.

Sugar samples were transferred to sterile 50% sucrose solution tubes. The tubes were then placed in the incubator and kept for several days at 34°C. When mould growth appeared a transfer was made to tubes of sterile agar, which were poured into plates. A similar series was made from all of the sugar samples on hand, using a molasses agar instead of the sucrose agar. Any decided difference in the appearance of the moulds developing upon the plates, made as above described, was deemed sufficient ground to regard the different growths as distinct species. This method of classification applied, of course, only to the use of the cultures in the preliminary experiments. All of the cultures were thereafter submitted to Dr. Charles Thom, of the Microbiological Laboratory of the Bureau of Chemistry, who very kindly offered to identify the cultures for us. The data on these mould cultures furnished us by Dr. Thom and the microanalyst of his department, Miss Margaret Church, are so valuable that it is deemed advisable to embody their reports in the text of this publication. The following are the data as transcribed from their respective letters:

Cultures I, IV and IX are Aspergillus niger.

Culture II is Aspergillus fumigatus.

Culture III is related to Aspergillus glaucus.

Culture VI is a blue Aspergillus.

Cultures VII and VIII are either floccose Aspergillus niger strain or else contaminated with some mould at present unrecognizable by us.

Cultures X and XIII are black or brown forms belonging to the Aspergillus niger group.

Culture XI is a blue-green form which we have gotten from several sources but have not satisfied ourselves as to name.

Culture XII is a sterile organism of orange color which we find constantly. Further than this, I know nothing

about it, since no fruiting has ever occurred, in spite of various attempts to induce it.

Culture XIV belongs to the Aspergillus glaucus series, probably being closest to A. repens.

The Culture III proved to consist of two organisms, a black and a green one. The green one proved to be a very interesting culture, which we have tentatively identified as Aspergillus pencilloides of Spegazzini. This was described by Spegazzini in a series of cultures obtained from sugar cane, in Argentina, some thirty years ago, and furnished a lot of interesting but troublesome data.

The authors suggest an interesting subject for investigation in connection with the inverting power of these mould cultures. The suggestion is as follows: *"If you could keep Nos. VII and VIII in a floccose and also in a purely fruiting condition during a series of transfers, and could establish a difference in the inverting power of either of these strains of Aspergillus niger according to the excess of mycelium or conidia which the cultures tended to produce, we would be decidedly interested."*

It is to be regretted that the lack of time for the further prosecution of this investigation prevented us from acting upon this valuable suggestion.

Referring to the description of the mould cultures, it will be noted that there were more duplications submitted of No. 1 than of any of the other cultures. That, of course, indicates the greater frequency with which this culture is found to occur in sugars, as compared with the other cultures.

What has been said with reference to the distribution of the bacterial cultures in sugars applies to an equal extent to the mould cultures. In many sugars, almost all of these cultures can be isolated. The difference in the infection of sugars with moulds seems also to be one of degree and not of kind. Sugars are rarely found to be entirely free from mould spores. It will be noted that the list of cultures obtained from sugars does not include a single one of *Penicillium glaucum*. This appears strange in view of Shorey's observation, as well as the general impression regarding the frequent occurrence of the mould in sugar products. A green mould is very frequently found on the

culture plates made from sugars, and for a long time the author regarded it as *Penicillium glaucum*. A study of its mode of spore formation, however, showed that it belonged to the aspergillus rather than to the penicillium group.

ACTION OF THE MOULD CULTURE UPON SUGAR SOLUTIONS.

The ability of any group of micro-organisms to cause the deterioration of sugars must depend at least upon the one condition that they can develop and can exercise this ability in highly concentrated sucrose solutions. To demonstrate this ability on the part of the mould cultures, the following experiments were conducted: Several series of solutions were made from a Cuban 96-test sugar. The solution varied in density from 52 to 72 Brix. Two hundred cc portions of these solutions were introduced into 300 cc Erlenmeyer flasks. The flasks and contents were then sterilized on three successive days and then inoculated with spores of the different cultures.

TABLE I.

Experiment III.
Table Showing Results of Experiment upon the Limits of Density in which Mould Cultures can Invert Sucrose.
One month Incubation.

Series	Cult No.	Name	Corrected Brix	Sucrose		% R. S.	Acidity 10 gm. Req. cc N/10	Loss in S. C.	Increase in R. S.
				S. P.	S. C.				
A	Cont...	.....	52.01	38.2	40.57	12.5	5.	—	—
"	I	Asp. niger...	53.69	1.3	12.3	25.0	—	28.27	12.5
"	II	Asp. Fumigatus...	53.21	21.2	27.78	34.7	1.2	12.79	22.2
"	VI	Blue Aspergillus.....	52.71	33.6	37.67	19.51	1.0	2.9	7.0
"	VII	.....	53.21	— .7	5.4	45.9	2.9	35.17	33.4
"	XI	.....	52.79	38.2	40.81	11.41	0.5	—	—
"	XIII	.....	52.67	4.2	14.25	42.10	2.0	26.32	29.6
"	VIII	Floccose Asp. niger...	53.08	0.9	17.5	44.4	2.2	23.07	31.9
"	XIV	.....	52.83	20.0	26 7	26.6	0.6	13.87	14.1

Experiment III---Continued.

TABLE II.

MOULD CULTURES

Series	Culture No.	Corrected Brix	% R. S.	Sucrose		Acidity 10grm. cc Req. N/10	Loss in S. C.	Increase in R. S.	B. — S. C.
				S. P.	S. C.				
B	Cont.....	61.08	12.1	51.5	53.39	0.5	—	—	7.69
"	I	62.55	34.7	19.1	28.19	2.8	25.20	22.58	27.85
"	II	61.26	16.0	41.2	45.27	0.9	8.12	3.88	15.99
"	VI	61.39	14.03	46.25	49.23	0.8	4.16	1.91	12.16
"	VII	61.39	43.10	17.9	26.97	2.1	26.42	29.98	34.42
"	VIII	61.98	42.10	17.0	26.63	2.0	27.36	29.98	35.35
"	XI	61.33	6.7	53.0	54.46	0.5	—	—	6.87
"	XIII	61.98	36.3	19.2	28.04	2.1	25.35	24.18	33.94
"	XIV	61.33	27.5	30.0	36.57	0.7	16.82	15.32	33.83

Experiment No. III.

TABLE III.

MOULD CULTURES

Series	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10gm. — cc N/10 Req.	Decrease in Sucrose	Increase in R. S.
			S. P.	S. C.				
C	Mould I.....	68.80	43.95	41.39	14.08	2.1	16.22	12.03
"	" II.....	69.20	51.20	47.23	6.09	2.9	11.38	4.04
"	" VI.....	68.83	64.50	57.61	2.51	0.9	1.00	0.46
"	" VII.....	69.00	52.70	48.35	12.60	2.8	10.26	10.55
"	" VIII.....	68.80	49.60	45.91	14.40	3.5	12.70	12.35
"	" XI.....	68.85	65.45	58.47	2.20	0.8	0.14	0.15
"	" XII.....	69.00	65.80	58.66	2.10	1.0	—	0.05
"	" XIII.....	68.83	50.30	46.76	12.30	3.3	11.85	10.25
"	" XIV.....	69.63	9.10	13.92	29.40	1.0	44.69	27.35
"	Mould VII— Yeast I —	67.35	63.60	57.42	0.29	2.2	1.19	—
"	Yeast.....	67.55	65.50	58.61	0.28	2.0	—	—
"	Control.....	68.75	65.40	58.61	2.05	0.9	—	—

Experiment III---Continued.

TABLE IV.

MOULD CULTURES

Series	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10gm. — cc N/10 Req.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.				
D	I.....	72.26	36.9	40.95	15.73	—	0.39	0.49
"	II.....	71.06	37.0	41.03	15.73	—	0.31	0.49
"	VI.....	72.02	37.2	41.10	15.73	—	0.24	0.49
"	VII.....	71.52	37.0	40.95	15.73	—	0.39	0.49
"	VIII.....	72.26	37.0	41.03	15.24	—	0.31	—
"	XIV.....	71.76	37.8	41.57	14.78	—	—	—
"	Control.....	72.50	37.3	41.34	15.24	—	—	—

RESULTS OF THE EXPERIMENTS.

A study of the above tables shows that with the exception of Culture XI all the cultures exercised a very strong inverting action in the solution below 72 Brix density. No inversion at all took place in that series, which indicates that the limit for inversion by these mould species is below that density. Culture X exercised no inverting action in any of the series. Cultures VII and VIII exercised the strongest inverting action of any of the cultures. The amount of inversion of sucrose in the sugar solution of 68 Brix is astounding, and indicates an ability on the part of these moulds to exercise a decided sucrose destroying power in the molasses films of certain types of sugars. If we study the results of Tables I and II we notice an interesting point in regard to the comparison between the amount of sucrose inverted and the increase in reducing sugar. In many cases we find that the increase in the latter is much greater than can be accounted for by the decrease in sucrose. Note, for example, Nos. VII and VIII in Tables I and II. This is possibly due to the formation of some reducing substance in addition to reducing sugars.

In connection with the rapid inversion by moulds of sucrose in solution of 69 Brix, it is interesting to note that the action of invertase is supposed to be very feeble in that concentration. O'Sullivan and Thompson[1] found that the rate of action of invertase decreased only slightly from an optimum concentration of 20 until 40% was reached. Beyond that point it was greatly enfeebled. Bokorny,[2] in working with compressed yeast, found that although these yeast could induce a vigorous fermentation in glucose solutions of 58.8%, yet in a similar concentration of cane sugar no fermentation took place. Only when a concentration of 74% was reached was the yeast inactive towards the former sugar. Bokorny would explain the suppression of invertase action in concentrated sucrose solution by attributing it to the lack of water necessary for the hydrolysis.

It seems evident that the invertase of moulds differs from that of yeast in its ability to retain its activity under conditions of high concentration.

[1]Jour. Chem. Soc. Trans. 57, 1890, 834.
[2]Beinflussing des Hefie Invertins durch Konzentrierte Zuckerlosungen Chem. Ztg. 1903, No. 90.

ARE TORULA AND MOULDS COMPETITIVE OR SYMBIOTIC IN THEIR ASSOCIATION IN SUGARS?

In a previous section of this report it was stated that although the torula in sugars could only invert sucrose in solutions of concentration of about 60 Brix, they are able to ferment glucose in solutions of very much greater density. This fact appears to offer a possibility for the deterioration of very high density sugar solutions by the combined activities of torula and mould. The possibility of this combined action depends, of course, upon whether these two groups of micro-organisms are competitive or symbiotic in their association. Referring to Table III we find that while the combined inoculation of Culture VII mould, and Culture I yeast, resulted in a slight loss of sucrose, this loss was much lower than that produced by the mould alone. In this case it appears that the activities of the yeast had greatly retarded the sucrose destroying power of the mould.

A similar experiment was next made upon a sugar solution of 71.5 Brix.

TABLE V.

Table Showing Action of Yeast and Mould Cultures on 71 Brix Molasses. 7-19-17

Samples	Treatment	Total Solids Brix	Sucrose		% R. S.	Acidity 10 gm. Req. cc
			S. P.	S. C.		
I	Control	71.58	37.2	40.76	14.4	9.50
II	Yeast Culture I	68.83	46.0	43.16	7.75	10.50
III	Mould II	68.40	45.6	42.24	7.60	9.50
IV	Yeast I / Mould I	69.15	46.4	43.47	7.54	9.00
V	Yeast I / Mould VII	68.20	46.0	43.07	8.09	10.00
VI	Yeast I / Mould VIII	68.60	45.6	43.38	8.63	10.50
VII	Yeast I / Mould VI	68.80	45.6	43.07	10.26	9.00

In the above table it will be seen that the action of the yeast in reducing the amount of total solids in the solution did not enable the mould cultures to invert any sucrose. On the contrary, the increase in purity of the inoculated solution was just as characteristic as if the torula had been used alone. Evidently the decrease in density of the solution brought about by the torula affords no advantage to the mould, but seems rather to suppress the activities of the latter by the accumulation of objectionable fermentation products.

PART II D.

THE CONDITIONS LIMITING THE RESPECTIVE ACTIVITIES OF THE THREE GROUPS OF MICRO-ORGANISMS IN SUGARS.

It is natural to suppose that the variation in the composition of sugars of different types would represent conditions of unequal suitability for the development and activities of the different groups of micro-organisms occurring therein. Under what conditions does the one or the other group gain the ascendancy? It is to the answering of this question that we will now direct our attention. From a superficial consideration of the subject, we might well conclude that the conditions differentiating the possible activities of the three groups of micro-organisms in sugars might be classified as follows:

(1) Degree of density of the molasses film.

(2) Its nutritive value.

(3) Its degree of acidity or alkalinity.

Let us consider these in their relative order. A series of solutions was made from 96-test sugar. The solutions varied in density from 37 to 72 Brix. The solutions were sterilized and inoculated with a culture of bacteria and a yeast, and also from a combination of the two. The results are given in the following table:

TABLE I.

Experiment on the Influence of Concentration of Sugars, upon the Differentiation of Action of Bacteria and Yeast.
Solutions made from 96-Test Sugar. One month Incubation.

Series	Treatment	Sucrose		Brix	R. S.	Loss in S. C.	Increase in R. S.	Decrease in R. S.
		S. P.	S. C.					
A 37 Brix.....	Cont.....	34.4	34.98	37.31	1.58	—	—	—
	Bact.....	− 6.6	2.69	38.52	29.46	32.29	27.88	.....
	Yeast.....	33.75	33.63	35.27	>.40	1.35	—	1.18
	Combined.....	4.6	6.96	35.72	19.50	28.02	17.92	—
B 43 Brix.....	Cont.....	39.70	39.90	43.34	2.15	—	—	—
	Bact.....	− 5.80	3.90	43.23	39.90	.36	37.75	—
	Yeast.....	38.20	38.11	40.60	>.84	1.79	—	1.31
	Combined.....	28.20	29.23	37.53	3.79	10.61	1.64	—
C 52 Brix.....	Cont.....	48.45	48.73	52.73	2.78	—	—	—
	Bact.....	−12.95	1.84	54.18	25.00	46.89	22.22	—
	Yeast.....	39.50	38.70	48.40	5.12	10.03	2.34	—
	Combined.....	37.45	36.83	47.45	5.65	11.90	2.87	—
D 60 Brix.....	Cont.....	55.80	55.94	60.83	3.57	—	—	—
	Bact.....	55.85	56.06	61.25	3.57	—	—	—
	Yeast.....	42.90	43.78	55.80	7.69	12.16	4.12	—
	Combined.....	41.45	42.09	55.55	7.89	13.85	4.32	—
E 72 Brix.....	Cont.....	66.30	67.31	72.30	4.54	—	—	—
	Bact.....	66.40	67.46	72.54	4.54	—	—	—
	Yeast.....	67.45	67.70	69.84	0.73	—	—	3.81
	Combined.....	67.75	68.10	70.69	0.69	—	—	3.85

From this table it will be seen that the destruction of sucrose by the bacterial cultures ended with the series C (52 Brix). The torula culture caused deterioration in the series D (60 Brix). Only in one case did the combined inoculation result in a greater destruction of sucrose than either of the other inoculations, and that was in series D. In the series in which the bacteria exercised any action the combined inoculation decreased the amount of sucrose destroyed, as compared with the solutions inoculated with bacteria alone. It is interesting to note that in both Series A and B the inoculation with yeast alone resulted in a decrease of reducing sugar similar to that which took place in higher density solutions. In fact, the results in these series are very similar to those of Series E. It is probable that the difference in the acidity of the solutions was responsible for these results. In the more dilute solutions less sugar was used, and the acidity of the solution was correspondingly lower than in the higher density solutions.

In the following table are the results of a similar experiment upon the comparative activities of a culture of bacteria and mould. The solutions were prepared and treated as in the previous experiment. The incubation period was two months.

TABLE II.

Experiment upon the Limit of Density in which Bacteria and Mould cause Sugar Deterioration.
Incubation two months.

Series	Treatment	Total Solids Brix	Sucrose		% R. S.	Acidity 10 cc req. N/5 X. 969
			S. P.	S. C.		
A 50 Brix.....	Cont.....	54.42	47.00	48.75	5.88	1.5
	Bact.....	55.50	46.90	48.53	5.88	—
	Mould.....	55.50	−14.40	3.69	50.00	12.5
B 60 Brix.....	Cont.....	64.08	55.50	57.48	6.66	0.5
	Bact.....	63.53	54.90	56.64	6.66	—
	Mould.....	64.50	− 6.15	10.20	55.50	3.0
C 65 Brix.....	Cont.....	67.05	57.85	59.45	6.40	0.5
	Bact.....	67.09	58.25	59.89	6.66	—
	Mould.....	67.50	2.95	18.40	48.70	3.0
D 70 Brix.....	Cont.....	69.34	61.10	62.74	5.26	0.4
	Bact.....	70.36	61.70	63.32	5.12	—
	Mould.....	69.56	9.90	24.48	46.50	3.5

In the above table it will be seen that the inoculation with the mould culture resulted in a marked deterioration of the Series D solution (69 Brix). No deterioration appeared to result from the use of the bacterial culture, even in Series A (55 Brix).

A third experiment was made, using white sugar syrups obtained from three plantations in the State, instead of solutions of 96-test sugar. This experiment was really intended to demonstrate the nutritive demands of the micro-organisms as well as the respective limits of density in which they could destroy sucrose. The syrups were sterilized and inoculated as in the previous experiment.

TABLE III.

Experiment upon the Relative Susceptibility to Deterioration of White Sugar Syrups from various Plantations.
Two months Incubation.

Series	Treatment	Total Solids Brix	Sucrose		% R. S.	Acidity 10 cc req. N/5 X. 969
			S. P.	S. C.		
A—Reserve.....	Cont.....	63.93	50.50	52.59	6.88	1.4
	Bact.....	64.26	50.50	52.49	7.14	1.5
	Yeast.....	58.28	42.30	44.28	5.12	2.4
	Mould.....	62.21	− 1.50	9.31	30.70	10.0
B—Wilbert.....	Cont.....	49.81	37.45	39.16	5.79	1.7
	Bact.....	51.46	38.80	40.62	5.88	1.6
	Yeast.....	40.98	− 3.00	2.92	40.00	3.0
	Mould.....	44.92	− 4.70	0.20	31.25	32.0
C—Ashland.....	Cont.....	60.23	48.00	49.90	5.00	1.6
	Bact.....	59.83	48.70	50.52	4.87	1.4
	Yeast.....	54.27	35.20	37.48	7.69	2.5
	Mould.....	57.88	− 3.10	6.13	39.21	14.0

The results of the experiment show that the mould culture exercises an immensely greater sucrose destroying power than did the cultures of the other micro-organism. The bacteria caused an appreciable loss of sucrose; the yeast culture caused a considerable loss even in the syrup of 63 Brix density.

From the standpoint of the nutritive value of the different syrups, no conclusion can be drawn, as the solutions deteriorated in accordance with what might have been expected from a consideration of their respective densities. We have, therefore, the following data upon the limits of density in which the respective groups of micro-organisms are able to destroy sucrose:

(1) Mould culture: Rapid deterioration in 69 Brix solution.

(2) Torula culture: Moderate deterioration in 64 Brix solution.

(3) Bacteria culture: Rapid deterioration in 52 Brix solution; no deterioration in 60 Brix solution.

THE RESPECTIVE NUTRITIVE REQUIREMENTS OF THE THREE GROUPS OF MICRO-ORGANISMS.

A solution was made of standard granulated sugar and distilled water. To this solution was added amounts of peptone varying from 0.2% to 1%. Three hundred cc Erlenmeyer flasks were then filled to two-thirds capacity with these solutions and sterilized. The flasks were then inoculated with a culture of bacteria and torula.

TABLE IV.

Experiment on Influence of Nitrogen upon the Action of Torula and Sugar Species. Incubation, one month.

Series	Treatment	Brix	Sucrose		% R. S.
			S. P.	S. C.	
A	Control	54.11	51.50	51.41	1.35
No Peptone	Bact.	52.51	51.50	51.57	1.37
	Yeast	51.96	50.00	50.04	1.73
	Bact and Yeast	53.54	50.90	50.63	1.60
B	Control	53.85	51.40	51.50	....
O. 2 Peptone	Bact.	53.37	50.00	50.64	....
	Yeast	51.92	48.00	47.78	3.20
	Bact. and Yeast	52.91	47.90	47.70	3.33
C	Control	55.14	51.70	—	2.16
O. 5 Peptone	Bact.	53.61	52.00	52.16	1.08
	Yeast	52.51	45.10	45.26	4.44
	Bact. and Yeast	51.68	46.00	46.21	4.21
D	Control	54.69	50.80	51.54	3.01
1% Peptone	Bact.	54.57	51.00	—	1.37
	Yeast	53.29	45.00	45.82	3.63
	Bact. and Yeast	52.79	43.05	43.74	5.33

From the above table it will se been that the amount of deterioration produced by the torula culture increased with the addition of peptone, until the addition reached 0.5%. There was no further increase beyond that point. There was no action produced by the culture of bacteria, as the density of the solution was probably beyond the maximum for its action.

In the next experiment a solution was made from plantation granulated sugar and distilled water. To this solution was added various nutritive ingredients. The solution was sterilized and inoculated as in the previous experiment.

TABLE V.

Experiment upon the Influence of Nutrient Salts upon the Deterioration of P. G. in Distilled Water.
Period of Incubation. one month.

Series	Treatment	Brix	Sucrose		% R. S.	Loss in Sucrose S. P.	Increase in R. S.	
			S. P.	S. C.				
Plant G.	Control.....	35.65	34.20	34.48	.909	—	—	—
+	Bact.....	37.00	− 1.8	2.55	33.3	32.40	32.391	Mould G.
Dist. Wt.	Yeast.....	35.95	32.80	33.23	2.35	1.40	1.441	—
	Combined B. & Y....	36.20	31.20	31.98	4.00	3.00	3.091	—
Plant G.	Control.....	35.95	33.70	34.25	1.48	—	—	—
+	Bact.....	36.30	23.50	—	5.18	10.20	3.70	Gummy
O. 5 KCL	Yeast.....	36.05	32.00	32.76	3.47	1.70	1.99	—
	Combined B. & Y....	36.30	32.25	32.63	3.12	1.45	1.64	—
Plant G.	Control.....	Lost	—	—	—	—	—	—
+	Bact.....	36.20	17.50	—	17.30	—	—	Gummy
O. 2%	Yeast.....	36.45	35.30	35.81	1.62	—	—	—
Na3 PO4	B. & Y.....	36.45	31.81	—	5.71	—	—	Gummy
Plant G.	Control.....	38.05	35.40	35.97	.909	—	—	—
+	Bact.....	38.05	2.60	—	40.00	32.80	39.091	Gummy
O. 5%	Yeast.....	34.30	10.90	11.56	23.52	24.50	22.611	—
Peptone	B. & Y.....	35.15	11.90	12.50	23.52	23.50	22.611	—
Plant G.	Control.....	37.25	35.30	35.49	—	—	—	—
Complete	Bact.....	37.25	4.40	—	33.33	30.90	—	Gummy
Formula	Yeast.....	31.04	3.90	—	21.62	31.40	—	—
	B. & Y.....	30.15	8.40	7.39	18.18	26.90	—	—
Stand. G.	Control.....	36.75	19.00	22.62	16.66	—	—	—
+	Bact.....	36.83	23.00	24.53	12.90	—	—	—
Dist. Wat.	Yeast.....	36.83	22.00	26.89	13.33	—	—	—
	B. & Y.....	36.28	19.90	25.06	13.79	—	—	—

In the above table it will be seen that the greatest deterioration produced both by bacteria and torula was in the series containing 0.5% peptone. In the series containing only sugar and distilled water there was a very high inversion of sucrose in the flask inoculated with bacteria. In this case, however, the solution contained a mould growth, due to contamination. The greatest deterioration produced by the torula culture was in the series containing all of the ingredients in the proportion prescribed in Smith's formula, viz:

(.5% Kcl .2% Na_3Po_4 .1% Peptone)

While the addition of these nutrient salts alone enabled the bacteria to cause a rapid destruction of sucrose, it did not produce the same effect upon the torula. Neither the bacteria nor the torula culture produced any appreciable effect upon the solution of standard granulated sugar in distilled water. An interesting point in connection with the above results is the large amount of inversion in the standard granulated control solution.

The solution contained no evidence of any fermentation changes, as it was perfectly clear, and no evidence of any microbial development could be found by microscopical examination. The inversion was due to the heating of the solution incident to sterilization. The solution of plantation granulated sugar did not undergo such an inversion, owing, no doubt, to its higher ash content. These solutions were sterilized by steam, at atmospheric pressure, so the amount of inversion in the control was entirely unexpected. This inverting action, however, is fully discussed by Von Lippmann.[1]

Another and much larger series of standard granulated solution was made, which included a larger number of nutrient additions than was used in the previous experiment. The solutions were prepared and inoculated as in the previous experiments.

TABLE VI.

Experiment upon the Deterioration of Standard Granulated Sugar in Distilled Water Solutions with addition of various Nutritive Compounds. Incubation one month.

Series	Inoculation	Total Solids Brix	Sucrose		R. S.
			S. P.	S. C.	
A					
Stand.	Control	36.39	27.35	28.71	7.27
Gran.	Bact.	35.88	29.35	25.81	5.40
Dist.	Yeast	36.14	29.00	30.22	5.12
Water	B. & Y.	36.48	29.35	30.52	5.71
B					
Stand.	Control	35.34	33.40	33.26	0.938
Gran.	Bact.	34.74	32.60	33.44	1.290
Tap	Yeast	36.39	33.40	33.13	0.966
Water	B. & Y.	35.88	33.50	33.36	0.854
C					
Dist.	Control	35.84	30.85	31.60	3.63
Water	Bact.	35.62	30.40	31.89	4.23
+ .01	Yeast	36.64	30.00	30.86	5.00
Peptone	B. & Y.	36.22	31.50	32.12	3.27
D					
Dist.	Control	36.14	34.00	34.15	1.13
Water	Bact.	35.37	22.55	Gum	12.90
+ .1%	Yeast	35.64	33.80	33.59	1.08
Peptone	B. & Y.	36.30	33.70	33.92	1.21
E					
.01%	Control	36.14	34.85	34.72	0.357
Sodium	Bact.	35.84	34.30	35.07	0.802
Phosphate	Yeast	35.92	34.40	34.99	0.869
	B. & Y.	36.37	34.80	34.04	0.588
F					
Dist.	Control	36.14	35.20	34.93	0.200
Water + .1%	Bact.	35.62	16.8	Gum	16.60
Sodium	Yeast	36.22	35.40	36.18	—
Phosphate	B. & Y.	36.37	34.85	34.71	0.227

[1]Chemie der Zuckerarten, Bd. II, p. 1228.

TABLE VI.

Experiment upon the Deterioration of Standard Granulated Sugar in Distilled Water Solutions with addition of various Nutritive Compounds.
Incubation one month.

Series	Inoculation	Total Solids Brix	Sucrose		R. S.
			S. P.	S. C.	
G .1% Peptone .1% Sodium Phosphate	Control	36.18	35.00	34.78	—
	Bact.	35.98	33.80	Gum	1.08
	Yeast	36.00	33.60	34.60	1.86
	B. & Y.	35.82	33.85	33.78	1.81
H .1% Peptone .1% Phosphate	Control	36.18	35.10	35.07	—
	Bact.	36.02	4.9	Gum	26.3
	Yeast	36.30	35.20	34.93	—
	B. & Y.	35.86	34.65	34.66	Trace
I .1% $CaSO_4$	Control	36.43	35.20	35.07	—
	Bact.	35.98	31.90	Gum	3.07
	Yeast	36.14	35.10	34.86	—
	B. & Y.	35.61	34.80	34.84	0.50
J .1% CaSO4	Control	36.18	35.10	34.84	—
	Bact.	35.98	33.20	Gum	2.66
	Yeast	36.07	35.20	34.93	—
	B. & Y.	36.15	33.00	Gum	2.00
K .1% Sugar Ash	Control	35.88	35.20	35.07	—
	Bact.	35.98	34.80	Gum	0.975
	Yeast	36.37	34.40	34.32	0.625
	B. & Y.	35.90	34.80	34.63	0.487
L .1% Sugar Ash	Control	36.18	35.20	35.07	—
	Bact.	35.18	32.80	Gum	2.94
	Yeast	36.15	35.20	34.93	—
	B. & Y.	35.90	33.30	Gum	2.66
M .1% Peptone + .1% Sugar Ash	Control	36.18	35.10	34.91	—
	Bact.	36.02	.64	Gum	25%
	Yeast	36.15	35.30	35.14	—
	B. & Y.	33.79	21.10	Gum	10.52
N .1% Peptone + .1% $CaSO_4$	Control	36.18	35.10	34.91	—
	Bact.	35.98	9.8	Gum	22.7
	Yeast	36.70	35.30	35.01	—
	B. & Y.	35.65	24.35	Gum	9.52

The results in the above table show that the addition of the ingredients to the standard granulated sugar solution did not result in as much deterioration from the action of the torula culture as in the experiment where plantation granulated sugar was used. On the other hand, the deterioration by the culture of bacteria was in many cases larger than in the preceding experiment. We find the greatest deterioration in Series H, which contained 0.1% peptone and 0.1% sodium phosphate. Series M, containing the same amount of peptone and 0.1% sugar ash, ranked next. Neither the bacteria nor the yeast culture could

induce any deterioration in the pure standard granulated solution. It is interesting to compare these results with those obtained in the preceding experiment on plantation granulated sugar. In that experiment the torula culture induced quite a considerable amount of deterioration in the solution of plantation granulated sugar in distilled water. It appears, therefore, that the addition of nutrient ingredients in the above experiment was in no case sufficient to make the nutrient value of the standard granulated solution equal to that of the pure plantation granulated solution for the development of the torula. From this it appears very probable that this question of nutrient value does exercise a considerable influence upon the keeping quality of white plantation sugars.

Another very interesting fact, which is brought out in the above experiment, is the relatively small deterioration produced by the combined inoculation as compared with the single inoculation with bacteria. It appears that in those cases the solutions were so deficient in nutrient value that the presence of the yeast resulted in depriving the bacteria of the necessary amount of food. The deterioration, therefore, is reduced as a result of a division of the nutrient ingredients of the solution. If we compare the amount of the inversion in the control of Series A with that of B, as well as with the other series, we find that even the presence of the mineral matter in the tap water acted as a partial buffer against this hydrolysis. The addition of the mineral matter in the other series, even in quantities as small as 1/100 of one per cent, entirely prevented the inversion of sucrose during the heating process.

In order to determine the influence upon the rate of sucrose destruction, of substances which would be likely to constitute the impurities of plantation granulated sugars, the following experiment was conducted. In this experiment, solutions were made from both plantation and standard granulated sugars and distilled water, as in the preceding experiments. The different series were treated as follows:

A. I. Plantation granulated sugar solution.

A. II. Same as above, with the addition of 1 cc of a 1:1000 molasses solution to each 100 cc of solution.

A. III. With 1 cc of a 1:1000 soil suspension to each 100 cc of solution.

A. IV. With 0.1% sodium phosphate.

Series B standard granulated sugar solution treated as in A.

TABLE VII.

Experiment on the Deterioration of Sugar Solutions from A-Plantation Granulated and Distilled Water.
Three weeks Incubation.

Series A	Inoculation	Corrected Brix	Sucrose		% R. S.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.			
I							
Plant.	Cont.	35.03	34.35	34.43	1.00	—	—
Gran.	Mould	36.88	− 8.40	1.4	30.00	33.03	29.0
and	Yeast	35.39	33.9	34.65	1.0	—	—
Dist.	Combined Inoc.	35.42	34.0	33.79	1.0	.64	—
Water	Bact.	35.73	31.4	Gum	5.0	2.9	4.0
II							
Plant. Gran.	Cont.	35.42	34.0	34.09	0.90	—	—
and 1 cc per	Combined	35.37	32.55	32.74	1.90	1.35	1.07
100 of 1-1000	Yeast	35.25	33.65	33.44	1.17	0.65	0.27
Mol. Sol.	Bact.	40.30	29.15	—	5.71	4.85	4.81
III							
Plant. Gran.	Combined	34.70	33.45	33.6	1.08	.3	0.28
and 1 cc per 100	Cont.	34.62	33.95	33.97	0.8	—	—
of 1-1000 Dirt	Yeast	34.62	33.65	33.36	0.95	.6	0.15
Suspension	Bact.	35.17	25.65	—	11.42	8.30	10.62
IV							
Plant. Gran.	Yeast	35.25	35.25	34.46	—	—	—
and .01%	Combined	34.77	34.77	32.70	2.28	—	—
Sodium	Cont.	Lost	—	—	—	—	—
Phosphate	Bact.	—	—	Gum	15.0	—	—

TABLE VII.---Continued

Experiment on the Deterioration of Sugar Solutions from B-Standard Granulated and Distilled Water.
Three weeks Incubation.

Series B	Inoculation	Corrected Brix	Sucrose		% R. S.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.			
I							
Stand. Gran.	Cont.	34.17	26.35	27.65	9.09	—	—
and	Mould	35.82	3.65	10.4	32.2	17.2	23.11
Dist. Water	Bact.	35.57	23.75	25.78	11.4	1.87	2.31
	Yeast	35.82	23.95	25.78	10.2	1.87	1.11
	Combined	35.82	23.55	25.78	12.5	1.87	3.41
II							
Stand. Gran.	Cont.	35.17	26.3	28.00	8.69	—	—
and 1cc1-1000	Bact.	35.42	23.6	26.37	12.2	1.63	3.51
Mol. Sol.	Yeast	35.42	24.0	26.83	12.2	1.17	3.51
	Combined	35.42	23.5	26.2	11.7	1.8	3.02
III							
	Cont.	35.42	25.4	27.7	10.52	—	—
	Combined	34.85	24.3	27.07	10.81	0.63	0.3
	Yeast	35.14	25.3	27.7	10.25	—	—
	Bact.	35.14	27.0	29.02	8.0	—	—
IV							
Stand. Gran.	Cont.	34.85	34.75	35.53	—	—	—
and .01%	Yeast	34.85	34.5	34.9	0.20	0.6	—
Sodium	Combined	34.85	34.7	35.25	0.40	—	—
Phosphate	Bact.	34.85	32.9	33.84	2.05	1.69	1.8

From the above table it will be seen that the mould cultures caused a heavy inversion in both of the untreated sugar solutions. The destruction of sucrose was more rapid, of course, in the plantation granulated solution. The bacteria caused a greater deterioration throughout the entire Series A than in B. The addition of the foreign matter to the solution in Series A increased greatly the action of bacteria. The action upon the yeast was not reflected in the results. With the exception of the deterioration caused by the mould cultures, there was no very pronounced sucrose destruction in any of the B series. It appears, therefore, that the amount of impurities added was insufficient to equalize the difference in the susceptibility of the two solutions to deterioration. It would be an entirely false assumption, however, to conclude that the addition to sugars of such extraneous matter as that used in the preceding experiments would necessarily tend to hasten their deterioration. On the contrary, it is quite likely that just the reverse would be true. While the substances added to the solution acted solely as a source of nutrition for the microorganisms when they are added to a sugar, they may serve to naturally increase the density of the moisture film and thus decrease the amount of available moisture.

In the following table are given the results of a two-months' storage of a plantation granulated sugar to which the following substances were added:

A Control.
B Distilled water.
C Tap water.
D 1:1000 molasses solution.
E 1:1000 dirt suspension.
F .01% sodium phosphate.
G 0.1% sodium phosphate.
H .01% calcium chloride.
I 0.1% calcium chloride.
J .01% calcium sulphate.
K 0.1% calcium sulphate.

TABLE VIII.

Table Showing the Influence of the Addition of Various Substances upon the Deterioration of Plantation Granulated Sugar.
Two months Incubation.

Sample	% Moist.	Sucrose		% R. S.	Factor	Dry Basis	
		S. P.	S. C.			S. P.	S. C.
A.....	.07	99.15	99.12	—	.082	99.21	99.18
B.....	.47	96.65	96.72	1.47	.140	97.10	97.17
C.....	.47	97.15	96.73	1.38	.164	97.60	97.18
D.....	.27	97.40	97.77	1.19	.103	97.66	98.03
E.....	.27	98.20	98.23	1.11	.150	98.46	98.49
F.....	.37	97.40	97.92	1.51	.142	97.76	98.28
G.....	.27	97.90	97.66	1.11	.128	98.16	97.92
H.....	.45	97.45	97.74	1.38	.176	97.89	98.18
I.....	.32	97.90	98.00	1.14	.152	98.21	98.31
J.....	.27	97.60	97.39	1.38	.112	97.86	97.65
K.....	.45	97.90	98.31	1.25	.214	98.34	98.75

The results in the above table show that the greatest deterioration took place in the series in which only distilled water was used. Where an ingredient was added in more than one quantity the deterioration was always greatest where the smaller amount was used.

From the results of our experiments upon the comparative nutritive requirements of the three groups of micro-organisms in sugars, we may conclude that their rank with regard to the possibility of their having these requirements satisfied in most any type of sugar is as follows:

(1) Mould.
(2) Bacteria.
(3) Torula.

THE DEGREE OF ACIDITY OR ALKALINITY OF THE MOLASSES FILM.

We will next consider the extent to which the reaction of the solution may control the activities of the different groups of micro-organisms in sugar. We have already seen that the bacteria in sugars have a very low tolerance of either acid or alkali. To determine this tolerance of acid and alkali by the torula the following experiment was conducted: To a white sugar syrup was added varying quantities of N/1 H_2SO_4, and N/1 NaOH. The addition was made after the syrup had been sterilized and

while it was still hot. The results are given in the following table:

TABLE IX.

Showing Results of Experiments upon the Influence of Reaction of Syrups upon the Action of Torula.
One month Incubation.
Ashland White Sugar Syrup.

Series	Corrected Brix	Sucrose		R. S.
		S. P.	S. C.	
A. .7 Acid Cult. I Yeast	53.37	30.25	34.12	8.5
Cult. II Yeast	53.45	30.4	34.62	8.33
A. .7 Acid Control	60.65	49.5	50.57	5.0
A. 1.7 Acid Culture I Yeast	53.05	23.6	27.9	12.5
1.7 Acid Culture II Yeast	53.38	24.3	28.86	13.3
1.7 Acid Control	61.61	48.3	49.8	6.45
1.5 Alkaline Culture I	54.90	38.1	41.73	2.89
1.5 Alkaline Culture II	54.78	38.3	40.95	2.85
1.5 Alkaline Control	60.16	49.9	50.7	5.00

From the above table it will be seen that the deterioration was much greater in the series having the highest acidity. It is interesting to note, however, that some inversion took place in the alkaline series. In the latter series we find a decrease in reducing sugar similar to that which takes place in very concentrated sugar solutions. It appears that in this series the inversion of sucrose was so retarded that the glucose was fermented more rapidly than it was formed.

If we try this same experiment, however, on a solution of higher density we will probably find that increasing the acidity does not extend the limit of density in which the torula can destroy sucrose.

TABLE X.

Part II D (3).
Experiment on the Influence of Acidity of Solution upon the Deterioration of Sugar Solutions Inoculated.
Culture I Torula.

Sample	Brix	Sucrose		R. S.	Acidity 10 cc req. N/10
		S. P.	S. C.		
A Control.....	70.65	58.60	60.35	9.09	.09
A Inoc.....	66.30	62.95	62.84	1.02	5.82
B Control.....	70.17	58.00	59.91	8.69	1.94
B Inoc.....	66.26	63.10	62.88	1.19	3.80
C Control.....	69.39	56.60	55.70	9.76	1.94
C Inoc.....	66.06	62.00	61.63	1.19	2.90

A. + .1 cc N/1 H_2SO_4 — 200 cc syrup.
B. + .3 cc N/1 H_2SO_4 — 200 cc syrup.
C. + .6 cc N/1 H_2SO_4 — 200 cc syrup.

The above table gives the results of an experiment on the deterioration of a syrup of varying acidities by a torula culture. It will be noted that the action of the torula was practically the same in all cases and that no inversion took place. Although the acidity was twice as great in Series B as in Series A, the action of the torula was still confined to the destruction of glucose. These results seem to indicate that increasing the acidity of the molasses film does not appreciably extend the maximum limit of density at which the sucrose destroying power of the torula is completely suppressed.

In the following tables are given the results of a similar experiment upon the influence of the acidity of the solution upon the destruction of sucrose by moulds.

TABLE XI.

Influence of Titration upon the Deterioration of Syrups.
A--.95 Acid.
Incubation one month.

Series A	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10 gm cc N/10 Req.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.				
A.....	Cont.....	62.40	59.2	59.45	1.19	1.0	—	—
0.95 Acid.....	Mould I.....	62.08	25.6	32.42	9.43	6.2	27.03	8.24
"	" II.....	62.61	22.9	30.46	10.20	3.5	28.99	9.01
"	" VI.....	62.03	57.7	58.05	2.71	1.0	1.40	1.52
"	" VII.....	62.28	23.9	32.22	7.46	4.0	27.23	6.27
"	" VIII.....	62.53	23.8	32.14	5.61	3.4	27.31	4.42
"	" XI.....	62.48	39.4	43.56	18.51	3.7	15.89	17.32
"	" XII.....	62.31	41.9	45.37	17.85	2.4	14.08	16.66
"	" XIII.....	62.33	25.5	32.48	Lost	4.7	26.97	—
"	" XIV.....	62.03	35.0	39.63	Lost	1.0	19.72	—
"	Yeast I.....	60.31	56.5	56.69	Lost	3.5	2.76	—

TABLE XII.

Influence of Titration upon the Deterioration of Syrups.
B- .55 Alk.
Incubation, one month.

Series B	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10 gm. cc N/15 Req.
			S. P.	S. C.		
.55 Alk.....	Mould I.....	61.89	22.9	24.37	23.80	6.9
"	" II.....	62.29	26.1	33.44	26.66	3.7
"	" VI.....	61.74	57.2	57.80	2.64	1.0
"	" VII.....	62.04	24.4	31.95	24.39	3.9
"	" VIII.....	62.38	21.0	22.87	26.31	4.7
"	" XI.....	61.92	51.3	53.15	3.49	1.0
"	" XII.....	61.87	58.1	58.64	2.33	0.8
"	" XIII.....	62.12	28.1	34.77	21.73	5.1
"	" XIV.....	62.67	3.7	9.10	20.00	0.9
"	Yeast I.....	59.07	55.3	55.97	0.64	3.7

A comparison of the results given in the two above tables shows that the inverting action of the mould cultures upon the sucrose in the different solutions was very pronounced in both of the series. In some cases a culture would cause a much higher inversion in Series A than in B, and with other cultures the reverse would be true. For example, observe the greater deterioration induced by Culture XIV in Series B than in Series A. Also the greater deterioration induced by Culture XII in Series A than in Series B. On the whole, therefore, it appears that, considering the group of mould cultures as a whole, they produced approximately the same amount of deterioration in both cases. This indicates that they can adapt themselves to a rather wide range of reaction. The torula culture used in the experiment induced some slight deterioration in both cases. In Series B the activities of the torula resulted in greatly decreasing the amount of glucose originally present in the solution. Unfortunately, the control flask in the series was lost, and also the flask inoculated with torula in the other series. So that we cannot compare the relative degrees of glucose destruction in the two cases.

It will be noted that the increase in acidity in the solution inoculated with torula is about equal to that resulting from the mould growth. It seems likely that it is due to this formation of acid that the torula is able to invert sucrose in an alkaline solution. The inversion probably does not begin until the fermentation of the glucose already present in the solution yielded sufficient acid to correct the alkalinity of the solution.

We may well assume from these experiments that moulds are not easily rendered inactive toward sucrose by slight changes in the reaction of the solution.

PART III.

THE INFLUENCE OF THE DENSITY OF THE MOLASSES FILM UPON THE DETERIORATION OF SUGARS.

In a previous part of this publication we advanced the theory that the validity of the Factor of Safety $\frac{W}{100-P} = .333$ depends upon the fact that it is an expression of a definite degree of density of the molasses film surrounding the sugar crystal. We will now subject this theory to a more critical study.

If we take any sugar and divide it up into several samples and allow these samples to absorb varying amounts of moisture, we should expect the rate of deterioration to vary with the moisture content. That is to say, we should expect the rate of deterioration to increase as the ratio of solid non sucrose to moisture decreased. The following table shows the results of an experiment, the purpose of which was to test this supposition. A Cuban 96-test sugar was taken and thoroughly mixed into eight equal portions. Samples A (1), (2) and (4) were allowed to lose some of their original moisture, while the remaining samples, with the exception of No. 3, were placed under moist chambers and allowed to take up moisture. A series of samples was then obtained, having a range of solid non sucrose moisture ratios from .207 to .747.

TABLE I.

Experiment on the Influence of Factor of Safety upon Deterioration of 96-Test Sugar. Incubation one month.

Sample	Original Factor	% Moist.	Sucrose		% R. S.	Dry Basis		Factor
			S. P.	S. C.		S. P.	S. C.	
A (1)....	.207	0.41	97.50	98.47	.543	97.90	98.87	.164
A (2)....	.220	0.57	97.25	98.09	.444	97.80	98.65	.207
A (4)....	.274	0.62	97.20	98.05	.520	97.80	98.66	.221
A (3)....	.392	0.90	97.00	97.28	.480	97.88	98.16	.300
A (6)....	.674	3.95	87.60	89.40	4.20	91.20	93.07	.318
A (5)....	.699	4.14	87.70	89.13	2.66	91.48	92.97	.336
A (7)....	.743	4.05	88.00	89.45	4.87	91.71	93.22	.337
A (8)....	.747	4.26	86.15	87.98	5.88	89.97	91.86	.307
ABOVE AFTER SIX MONTHS								
A (1)....	.207	0.52	97.5	97.92	.555	98.00	98.42	.115
A (2)....	.220	0.61	96.8	97.12	.537	97.39	97.71	.190

A glance at the above table shows that no appreciable deterioration took place in any of the samples having a factor of less than .674. The increase in the moisture contents of A (6) over A (3), the next lower in the series, was more radical than was desired, since it would have been better to have had several samples intervening between the two. However, this does not obscure the real purpose of the experiment, which is to interpret the results in the light of the probable density of the molasses film in each case. In A (3) we have the original sample with its normal moisture content. Let us assume that the molasses on this sugar is of 75 Brix density, which is more likely an overestimate than an underestimate of its density. Sample A (6) has absorbed over three times its original amount of moisture, so the density of the film is probably not more than one-half its original density. It would probably be incorrect to assume that the molasses films of the crystal could be diluted to the full extent of the moisture absorbed without it taking some of the sucrose crystal into solution. Nevertheless, the film must be considerably diluted as a result of moisture absorption, for otherwise the increase in moisture would not affect the deterioration of the sugar. We must assume, therefore, that the molasses film surrounding a sugar crystal can exist in a more dilute state in the form of a film than when an equal amount of water is added directly to sugar. In other words, the crystal seems to offer a certain resistance to the solvent action of the film moisture. If we examine the table showing the analyses of samples A (1) and (2) after six months' storage we will find that no deterioration took place. It appears that in those samples the molasses films were of too great a density for the development of microorganisms.

For the purpose of determining the influence of the density of the molasses film upon sugar deterioration it is preferable to use sugars with films of known composition. It thus becomes necessary to make sugars from magmas, in which the ratio of sugar crystals to molasses is known. With this idea in view, a series of sugars were made in the laboratory. A standard granulated sugar furnished the crystals, and a series of syrups were made from varying mixtures of a white sugar syrup and a

Louisiana blackstrap molasses. The analyses of the original syrup, the finished sugars and the resulting molasses are given in the following tables, respectively:

TABLE II.

Experiment on the Deterioration of Laboratory made Sugar and Molasses.

ORIGINAL ANALYSIS OF SUGAR

Sample	Proportion of Mixture	% Moist.	Sucrose		R. S.	Dry Basis		Factor
			S. P.	S. C.		S. P.	S. C.	
I	White from 99 purity.....	.61	99.1	99.24	—	99.7	99.84	.677
III	¾ White Sugar ¼ B. S.....	.85	98.2	98.6	7.25	99.04	99.44	.472
IV	½ W. S. ½ B. S.....	.85	97.7	98.4	0.43	98.53	99.23	.369
V	⅜ W. S. ⅝ B. S.....	1.04	97.00	97.26	0.80	98.01	98.27	.346

TABLE III.

ORIGINAL ANALYSIS OF MOLASSES

Sample	Proportion of Mixture	Brix	Sucrose		R. S.	Purity	Factor
			S. P.	S. C.			
I	White from 99 purity.....	69.16	68.45	68.25	—	98.9	.977
III	¾ W. S. ¼ B. S.....	72.17	52.00	54.31	8.00	72.05	.579
IV	½ W. S. ½ B. S.....	73.82	47.20	49.50	9.5	63.90	.495
V	⅜ W. S. ⅝ B. S.....	74.55	40.00	43.80	12.9	53.6	.440

TABLE IV.

ANALYSIS OF SYRUPS USED IN MAKING SUGAR

Sample	Proportion of Mixture	Brix	Polarization	Purity	Factor
I	Pure White solution.....	67.64	67.00	99.05	.977
III	2 prts. sug. sol., 1 pt. B. S..	71.43	53.20	74.47	.610
IV	1 part I, 1 part B. S.....	73.54	48.00	65.27	.508
V	1 part I, 2 parts B. S.....	74.78	44.00	58.80	.450

A comparison of Tables III and IV shows that the Brix of the molasses is slightly higher in certain cases than that of the corresponding syrups. This was due to the unavoidable passage of some fine grains of sugar through the centrifugal screen.

In the following table are given the results of the analyses of sugars and molasses after an eight-month storage period:

TABLE V.

Experiment on Deterioration of Laboratory made Sugar and Molasses.---Continued.

ANALYSIS OF SUGAR AFTER EIGHT MONTHS

Sample	Proportion of Mixture	Sucrose		% Moist.	Dry Basis		R. S.	Loss in S. C.	Increase in R. S.	Factor
		S. P.	S. C.		S. P.	S. C.				
I	White from 99 purity.....	98.5	98.68	0.30	98.79	98.97	.510	—	—	.200
III	⅔ W. S. ⅓ B. S.....	96.65	96.85	0.85	97.48	97.68	1.58	1.29	1.07	.253
IV	½ W. S. ½ B. S.....	96.0	97.26	0.90	96.87	98.14	1.14	0.83	0.63	.225
V	⅓ W. S. ⅔ B. S.....	96.6	96.96	1.10	97.67	98.03	1.06	0.94	0.55	.323

The results given in the above table show that the rate of deterioration of the different sugars is, with one exception, what was to have been expected from their respective "factors of safety." The exception is with the white sugar, which might have been expected to deteriorate. In that case it is quite likely that although the ratio of solids non sucrose to moisture was low, the actual moisture present was insufficient for the development of micro-organisms. Regarding this condition we will have more to say in a subsequent portion of this publication.

TABLE VI.

ANALYSIS OF MOLASSES AFTER EIGHT MONTHS

Sample	Proportion of Mixture	Brix	Sucrose		R. S.	Loss in S. C.	Increase in R. S.
			S. P.	S. C.			
I	White from 99 purity.....	69.64	50.7	54.39	13.3	10.37	8.76
III	⅔ W. S. ⅓ B. S.....	70.49	43.2	46.18	14.9	7.24	5.34
IV	½ W. S. ½ B. S.....	71.09	37.6	42.31	16.12	4.08	5.14
V	⅓ W. S. ⅔ B. S.....	71.38	27.60	35.07	20.8	2.67	5.5

In the above table are given the results of the analyses of the molasses from the sugars after a similar period of storage. It will be observed that the rate of deterioration of the molasses samples is in inverse proportion to their density, and increases directly with the decreasing ratio of solids non sucrose to moisture.

The deterioration of these molasses samples with densities of 73 and 74 Brix does not find an exact parallel in the results of any of our experiments upon the inoculation of syrups and molasses. However, it is very probable that sufficient moisture was absorbed and held as a surface film to form a zone of suitable density for the development of micro-organisms. The molasses samples were kept in jars, which were provided with metal covers, but these did not form an entirely airtight cover. This seems all the more likely when we consider that the storage period was eight months. While the moisture content of the sugars did not vary appreciably during the storage period, yet it was possible that they might have absorbed some moisture during the period intervening between the time that they were made and the time of the analysis.

In the next experiment it was decided to avoid all possibility of the sugars absorbing moisture from the air, in order to determine whether this moisture is essential to the deterioration of sugars in which the ratio of solids non sucrose is less than three to one. A question that has often been raised in connection with the subject of sugar deterioration is whether or not the molasses films of sugars are homogeneous. If sugar deterioration depends, as some would argue, upon the fact that the outer periphery of the molasses film is much more dilute than the interior, then the deterioration of two sugars with the same factor might be different in case one had absorbed moisture and the other had not. We should, therefore, be able to demonstrate the truth of this theory by making sugars with films of known composition and storing them in sealed containers, and comparing them with sugars which have attained the same factor by the absorption of moisture. An experiment, therefore, was conducted as follows: A coarse standard granulated sugar was used for the experiment. The series of sugars were made with the use of the following syrups:

A. Final molasses alone.
B. $\frac{5}{6}$ final molasses + $\frac{1}{6}$ white sugar syrup.
C. $\frac{2}{3}$ final molasses + $\frac{1}{3}$ white sugar syrup.
D. $\frac{1}{2}$ final molasses + $\frac{1}{2}$ white sugar syrup.

The following table gives the results of the analyses of the resulting molasses:

Series	Corrected Brix	R. S.	Sucrose Clerget	Reducing Sugar
A	75.3	29.6	37.42	19.2
B	74.29	36.30	41.25	16.4
C	72.39	41.0	44.69	14.2
D	70.14	46.50	49.88	9.6

After drying the sugars in a laboratory centrifugal each sample was thoroughly mixed and divided into several portions. One portion was immediately placed in a glass jar with ground glass stopper and sealed with paraffin. Another portion was analyzed, and the remaining portion placed in moist chambers and allowed to absorb moisture. Samples A, B, C and D are the original samples kept in sealed containers. Samples A. I, A. I (1), B. I, C. I and D. I are the samples that absorbed moisture. The following table gives the per cent of molasses that the different sugars retained, as calculated from their respective moisture contents; also the per cent of reducing sugar found by analysis, the amount calculated from the calculated molasses, and also the calculated sucrose in the film:

Sample	Calculated % Molasses	R. S. Found	Calculated R. S.	Calculated Sucrose
A	4.44	.98	.84	1.66
B	3.38	.55	.69	1.39
C	2.89	.41	.51	1.29
D	4.34	.411	.52	2.16

TABLE VII.

Analysis of Sugars made in Laboratory.
Incubation three months.

Sample	Factor	% Moist.	SUCROSE Dry Basis S. P.	SUCROSE Dry Basis S. C.	% R. S.	Loss in S. C.	Increase in R. S.
A—original	.314	1.1	97.57	97.73	.98	—	—
A—3 mo. Storage	.400	1.2	98.17	98.22	1.04	—	.06
AI—original	.383	1.48	97.57	97.73	.98	—	—
AI—3 mo. Storage	.361	1.32	97.63	97.62	1.20	.11	.22
AI (1) original	.395	1.55	97.57	97.73	.98	—	—
AI (1) 3 mo. Storage	.333	1.32	97.28	97.19	1.18	.54	.20
B—original	.328	.87	98.21	98.62	.69	—	—
B—3 mo. Storage	.280	.85	97.83	98.22	.95	.40	.26
BI—original	.362	1.27	98.21	98.62	.69	—	—
BI—3 mo. Storage	.340	1.02	97.99	97.85	1.04	.77	.35
C—original	.333	.80	98.38	98.55	.51	—	—
C—3 mo. Storage	.275	.80	97.88	98.14	.98	.41	.47
CI—original	.485	1.49	98.38	98.55	.51	—	—
CI—3 mo. Storage	.350	1.40	97.36	97.51	1.35	1.04	.84
D—original	.464	1.30	98.48	98.59	.52	—	—
D—3 mo. Storage	.367	1.27	97.79	96.72	1.16	1.87	.64
DI—original	.544	1.76	98.48	98.59	.52	—	—
DI—3 mo. Storage	.353	1.52	97.06	97.28	1.38	1.31	.86

A—Made from coarse granulated sugar + Final molasses.
B—Made from coarse granulated sugar + 5/6 Final molasses, 1/6 White S. Syrup.
C—Made from coarse granulated sugar + ⅔ Final molasses, ⅓ White S. Syrup.
D—Made from coarse granulated sugar + ½ Final molasses, ½ White S. Syrup.

The results given in the above table show that of the samples of the sugars which were prevented from absorbing moisture only one deteriorated appreciably in storage, and that was "D" with a factor of .464. Series C, with a factor of .333, held its polarization. If we take C. I and D for comparison we have two sugars with approximately the same factors, but with the difference that the former has absorbed .6 per cent of moisture and the latter none. If absorbed moisture creates especially favorable conditions for deterioration, then the deterioration in C. I should be greater than in D. The results show no such difference. In fact, the deterioration in the two cases is as nearly equal as we could expect from the close agreement of the solid non sucrose moisture ratios. While the other samples do not agree as closely in their factors and therefore are not as favorable for comparison, yet the results in no single instance indicate that any especially favorable condition for sugar deterioration is created by moisture absorption beyond what is indicated by the solid non sucrose moisture ratio. If we refer to the density of the molasses from this sugar we find that molasses D had a Brix of 70.14. The next in the series had a density of 72.39. Of the original sugars,

only D deteriorated, which rather tends to confirm our experiments on the limit of density for sucrose destruction by the group of micro-organisms in sugars.

That the rate of deterioration of a sugar is to a large extent comparable to the deterioration of a molasses or syrup of a composition similar to the molasses film of such a sugar is shown by the following experiment. In this experiment one of the plantation white sugar syrups, No. A, used in Part II, was employed. A coarse standard granulated sugar was used in making up the magma. The sugar was dried in the laboratory centrifugal as in the previous experiments. The syrup used was divided into two portions, one of which was neutralized with lime, the other allowed to retain its original acidity, which was such that 1.3 cc of N/10 NaoH was required for each 10 grams.

TABLE VIII.

Analysis of Laboratory Sugars Sterlized in Autoclative 15′ at 15 lbs. pressure Reserve White Sugar Syrups used @ 63 Brix.

Sample	Treatment	% Moist.	Sucrose		R. S.	Dry Basis		Factor
			S. P.	S. C.		S. P.	S. C.	
4	Acid Sugar Control.....	.85	98.30	98.49	.27	99.14	99.33	.500
2	Acid Sugar Inoc. Bact.....	.42	97.50	98.04	.66	97.91	98.45	.168
3	Acid Sugar Inoc. Mould.....	.97	96.50	97.12	1.60	97.44	98.06	.277
1	Acid Sugar Inoc. Yeast.....	1.05	98.35	98.61	.2	99.39	99.65	.636
7	Neutral Control.....	.92	98.45	98.69	.5	99.36	99.60	.593
6	Neutral Inoc. Bact.....	.35	96.30	96.92	1.90	96.44	97.06	.094
8	Neutral Inoc. Mould.....	.87	96.80	97.38	1.30	97.64	98.22	.271
5	Neutral Inoc. Yeast.....	.70	98.70	98.99	.31	99.39	99.68	.538
11	Neutral Abs. Moist Control.....	1.15	98.15	98.26	.50	99.29	99.40	.621
10	Neutral Abs. Moist Inoc. Bact.....	1.50	97.40	97.50	.6	98.88	98.98	.576
12	Neutral Abs. Moist Inoc. Mould.....	1.20	94.76	95.23	2.33	95.91	96.38	.229
9	Neutral Abs. Moist Inoc. Yeast.....	1.02	98.00	98.15	.41	99.00	99.15	.510
13	Acid Sugar Abs. Moist Inoc. Bact.....	1.02	98.15	98.42	.28	99.16	99.43	.551

A study of the above table shows that with the exception of Sample No. 6, only the samples inoculated with moulds deteriorated. The deterioration caused by mould was greater in the acid than in the neutral sugar, but greatest of all where the neutral sugar was allowed to absorb moisture, in which case it acquired a factor of .621. It appears strange at first sight that the bacteria should have caused a deterioration of this sugar, when the same culture caused no deterioration in the original syrup. (See Table III, Part II-D.)

A microscopical examination of this sugar sample, together with plates made from it, showed that the apparent inconsistency in results was due to a contamination with mould. Both the microscopical examination of the sugar and the plates made from it showed a predominance of mould in the sample. Neither the bacteria nor the yeast cultures seemed able to cause any appreciable deterioration of the sugars. To determine the action of the bacteria upon a sugar with a lower ratio of solids non sucrose to moisture, the following experiment was conducted: A white sugar magma was made in the laboratory and dried as in the previous experiment. The syrup used in making this magma was divided into two portions, one of which was neutralized with lime and the other acidified with SO_2. The Brix of this syrup was 65.7.

TABLE IX.

Table Showing Results of Inoculation of Laboratory Made Sugar with Culture of Bacteria.
Incubation period one month.
A. White Sugar.

Series	Reaction	Treatment	Date	S. P.	S. C.	Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Moist.	Original Analysis Ratio Solid Sucrose to Moist.
								S. P.	S. C.		
I	Neut.	Cont.	6/21	97.8	98.07	1.22	.554	99.00	99.28	1.57	.747
		Inoc.	"	97.55	97.72	1.55	.632	99.08	99.25	1.40	.721
	Acid	Cont.	"	97.9	98.30	1.19	.566	99.07	99.48	.92	.557
		Inoc.	"	98.0	98.22	0.92	.460	98.90	99.13	1.17	.624
II	Neut.	Cont.	"	98.3	98.23	0.71	.417	99.00	98.93	.42	.428
		Inoc.	"	97.8	98.07	1.05	.477	98.82	99.11	.60	.660
	Acid	Cont.	"	98.3	98.46	0.82	.482	99.11	99.27	.55	.426
		Inoc.	"	97.9	97.99	1.08	.514	98.96	99.05	1.10	.604
III	Neut.	Cont.	"	97.4	97.75	1.91	.734	99.29	99.65	1.23	.694
		Inoc.	"	97.6	97.91	1.80	.750	99.38	99.70	1.57	.744
	Acid	Cont.	"	96.8	96.81	2.11	.659	98.88	98.89	1.55	.682
		Inoc.	"	97.8	97.90	1.63	.740	99.42	99.52	1.35	.648

ANALYSIS OF ORIGINAL SUGAR

Series	Sucrose		Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Molasses	
	S. P.	S. C.			S. P.	S. C.	Titre	Brix
Acid	98.5	98.6	0.77	.513	99.26	99.36	1.1 cc	65.76
Neutral	98.6	98.62	0.85	.614	99.44	99.46	Neut.	65.76

The results in the above table show that no appreciable deterioration took place in any of the inoculated samples, in spite of the fact that the solid non sucrose moisture ratio was considerably under the point of safety. However, this was what should have been expected in consideration of the density of the syrup used in making the sugars and which constituted their films. A similar experiment was next tried on a laboratory-made 96-test sugar. The molasses used in making the magma was, of course, much higher in density than that used in the previous experiment. The reaction of the molasses was adjusted as in the previous experiment. The sugars were divided into three lots and allowed to absorb different amounts of moisture. In some instances the moisture content of the sugar was over two per cent.

TABLE X.

Table Showing Results of Inoculation of Laboratory Made Sugars with Culture of Bacteria.
B. 96-Test Sugar.

Series	Reaction	Treatment	Date	S. P.	S. C.	Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Moist.	Original Analysis
								S. P.	S. C.		Ratio Solids Non Sucrose to Moist.
I	Neut.	Cont.	6/23	97.3	97.27	1.37	.507	98.65	98.62	1.65	.563
		Inoc.	"	97.4	97.67	2.14	.823	99.52	99.80	1.97	.609
	Acid	Cont.	"	96.8	96.88	1.32	.412	98.09	98.17	1.65	.563
		Inoc.	"	97.2	97.20	1.26	.450	98.44	98.44	1.47	.546
II	Neut.	Cont.	"	96.2	96.25	1.93	.507	98.09	98.14	1.87	.595
		Inoc.	"	96.4	96.84	1.49	.413	97.85	98.30	1.85	.612
	Acid	Cont.	"	97.1	97.20	1.06	.365	98.14	98.24	1.22	.409
		Inoc.	"	97.3	97.27	0.90	.333	98.18	98.15	1.12	.476
III	Neut.	Cont.	"	96.5	96.92	1.66	.474	98.12	98.55	1.92	.603
		Inoc.	"	96.3	96.45	2.06	.556	98.32	98.47	2.50	.670
	Acid	Cont.	"	96.5	96.64	1.91	.545	98.37	98.53	1.90	.612
		Inoc.	"	96.8	97.15	1.52	.475	98.29	98.64	2.00	.626

ANALYSIS OF ORIGINAL SUGAR.

Series	Sucrose		Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Molasses	
	S. P.	S. C.			S. P.	S. C.	Titre	Brix
Acid	98.2	98.28	0.56	.311	98.75	98.83	10 cc	74.49
Neutral	98.0	98.35	0.69	.345	98.68	99.03	0.4 cc	74.49

The results of the above table show that no deterioration took place in any of the inoculated samples. The conditions in both acid and neutral samples were alike unfavorable for the action of the bacteria. Evidently the molasses was of too high a density to allow the bacterial development to take place.

We have already referred, in a previous section of this report, to the characteristic manner in which torula destroy the reducing sugar in raw sugars and thereby increase their polarization. The following table gives an example of this selective action of the torula cultures upon reducing sugars:

TABLE XI.

Experiment on Deterioration of Sugars Inoculated with Torula Cultures.

Inoculation	Sucrose		Reducing Sugars	Moist.	Period of Inoculation days	Moisture Free Basis		Decrease in S. C.	Increase in R. S.	Factor
	S. P.	S. C.				S. P.	S. C.			
No. 7 96 Control	92.6	92.60	1.42	4.08	30	96.56	96.56	—	—	.550
Cult. I Yeast	94.0	93.71	0.41	4.36	"	98.23	97.98	+ 1.42	− 1.01	
Cult. II	93.6	93.96	0.392	4.33	"	97.84	98.21	+ 1.65	− 1.03	
Cult. III	93.5	93.91	0.40	4.75	"	98.16	98.59	+ 2.03	− 1.02	
No. 8 Control	90.1	91.19	3.88	5.12	"	95.00	96.20	—	—	—
No. 2 Cult. I	92.0	91.97	2.67	5.39	"	97.30	97.30	+ 1.10	− 1.21	—
Cult. II	91.8	92.02	2.75	5.58	"	97.30	97.50	+ 1.30	− 1.13	—
Cult. III	91.3	91.54	2.98	5.23	"	96.40	96.70	+ 0.50	− .90	—

In the above table it will be noted that in almost every case the inoculation of the sugar with torula resulted in a marked decrease in the per cent of reducing sugars remaining in the sample at the end of the experiment.

EXPERIMENT No. XII.

B. III.

Sugars Inoculated. Absorbed Moisture.

Series	% Moist.	Sucrose		Dry Basis		Original Factor	Final Factor	% R. S.	Inoculation
		S. P.	S. C.	S. P.	S. C.				
III									
B. III (4)....	2.79	95.1	95.76	97.82	98.48	.699	.569	.691	Yeast
B. III (1)....	1.67	96.4	97.08	98.03	98.71	.723	.463	.684	Bact.
B. III (2)....	2.72	94.1	94.68	96.73	97.31	.682	.444	1.412	Mould

The above table gives the results from an experiment on the inoculation of the same sugars as used in the previous experiment with a culture of bacteria, yeast and mould, respectively. Only the sample inoculated with mould deteriorated during the incubation period of one month. It will be noted that the ratio of solid non sucrose to moisture was low in all cases. The solid non sucrose moisture ratio was least favorable to deterioration in the sample inoculated with mould.

PART IV.

PRACTICAL APPLICATION OF THE RESULTS OF THE INVESTIGATION TO THE CONDITIONS EXISTING IN THE CANE SUGAR INDUSTRY.

We will now endeavor to ascertain the extent to which the principles established by our investigation are susceptible of direct application to industrial conditions as a preventive measure against losses from sugar deterioration. The measures for the prevention of sugar deterioration may, for convenience, be classed under three distinct heads:

(1) The manufacture of sugars which conform in composition to the best standards of keeping quality.

(2) Taking precautionary measures to prevent the excessive infection of sugars with deteriorative microorganisms.

(3) Providing suitable storage conditions for sugar.

When we consider the composition of sugar with special reference to its influence upon their deterioration, we are irresistibly drawn to the conclusion that the most important factor in this particular connection is the density of the molasses film surrounding the crystal. It is upon this fact that the validity of the solid non sucrose moisture ratio seems to depend. Since the density of the film is the all-important factor in sugar deterioration, it is of importance to find out how this principle can be utilized in factory practice. Here we have, for example, the practice of washing sugars in the centrifugal up to higher polarization tests. This practice results necessarily in diluting the molasses film and therefore lowers the solid non sucrose moisture ratio. Therefore, a washed sugar deteriorates with a much lower moisture content than the same sugar would otherwise have done. The validity of the "Factor of Safety" establishes the principle that the safety of a sugar from deterioration is entirely independent of the amount of molasses it contains. In other words, it is the composition of the molasses film, and not the crystal that it surrounds, which determines the susceptibility of a sugar to deterioration. However, it is of course obvious that the extent

to which a sugar can lose in polarization from deterioration depends on the amount of molasses surrounding the crystal. This fact is very clearly shown in the following table, which was prepared by Mr. F. B. Brenneman, superintendent of the Rio Cauto factory, who has kindly authorized me to use it. The explanation of the table and the discussion of it is quoted directly from an article submitted to the writer by the above-named author.

TABLE I.

Polarization	85 Brix 38.25 Sucrose 45 Purity			83.75 Brix 41.88 Sucrose Molasses 50 Purity			82.5 Brix 45.375 Sucrose 55			81.25 Brix 48.75 Sucrose 60			80 Brix 52% Sucrose 65		
	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor
94.00	9.71	1.46	.243	10.32	1.68	.280	10.98	1.92	.320	11.71	2.20	.366	12.49	2.50	.417
94.5	8.91	1.34	.243	9.46	1.54	.280	10.07	1.76	.320	10.73	2.00	.364	11.45	2.29	.417
95.00	8.10	1.215	.243	8.60	1.40	.280	9.15	1.60	.320	9.76	1.82	.364	10.41	2.08	.416
95.50	7.29	1.094	.243	7.74	1.26	.280	8.23	1.44	.320	8.78	1.64	.364	9.37	1.87	.416
96.00	6.48	.972	.243	6.88	1.12	.280	7.32	1.28	.320	7.80	1.46	.365	8.33	1.66	.415
96.50	5.67	.85	.243	6.02	.98	.280	6.41	1.12	.320	6.83	1.28	.366	7.29	1.46	.417
97.00	4.86	.729	.243	5.16	.84	.280	5.492	.96	.320	5.85	1.10	.366	6.25	1.25	.417
97.5													5.20	1.04	.416
98.0													4.16	.83	.415

This Table Calculated — Assuming sugar grains 100% Sucrose.

The resulting sugars being pure grains coated with molasses of varying purities: Then a sugar Pol. 94 $-\frac{100.}{90.29}$ 9.71% Mol. grains $\times$ 38.25 $= \frac{3.714}{90.29}$ $= 94.004$ P. of sug.

The Brix, and Sucrose to correspond to Molasses of varying purities assumed.
No water used in centrifugals.

A very interesting and important practical point is mentioned in the discussion of the preceding table by the author. The point in question is the relative keeping qualities of two sugars with the same "factor of safety," but with different polarization. The margin of safety is greater, as Mr. Brenneman points out, in the sugar with the lower polarization, since it can absorb more moisture without attaining a dangerously low ratio of solids non sucrose to moisture.

According to the formula given by Deerr & Brown used in Australia, the sugar may be considered safe if $\frac{\text{Moisture \%}}{100 - \text{Pol.}} =$ *33 1/3.*

If we consider that grains of sugar are practically pure, then if sugar was dried in centrifugals without washing, the moisture and the factor 100 — Pol would be the moisture and non-sucrose of the molasses; therefore, the factor of safety would remain the same regardless of per cent of molasses left in sugar, since the moisture and non-sucrose would increase or decrease in same proportion.

Reasoning along this line, sugar made which yields a high purity molasses would not have as good keeping quality as sugar yielding lower purity molasses, since with the higher purity molasses surrounding crystals the moisture would be in larger proportion to the non-sucrose, where polarization of sugar is the same.

The following table shows this approximately:

"In the preceding table it is shown that in centrifugalling without washing, that sugar made giving off a molasses of a certain purity, will have same factor of safety regardless of polarization or degree of completeness which molasses is removed."

"That the factor of safety is better with sugars giving low purity molasses than where high purity molasses is given off.

That a 96 test sugar from high purity massecuite will be required to retain a larger per cent of molasses and a larger per cent of moisture than where lower purity is used, and factor of safety not as good."

"In working in tropics where we have high purity syrups we find two methods of pan work used:"

System I—Making first massecuite nearly pure syrup—say, 80-85 purity. Mol. 60-65. Mixed massecuite 72-75 purity. Mol. 45-50. Low grade massecuite 60 purity. Mol. exhausted.

System II—First massecuite 75-78 purity. Mol. 54-55. Second massecuite 60 purity. Mol. exhausted.

"For a number of reasons System No. 1 is preferred:

Molasses given absolute change: Possible thus to get low purity mixed molasses to take back on the third grade M. C.

As to sugar made, that from high purity M. C. will have high polarization and low moisture if grain is large and centrifugal speed good. But as shown in table, factor is bad."

"That from low purity M. C. will have lower polarization, though if polarization of the other sugar is high enough this sugar may be dried without water and mixed with higher sugar giving desired polarization. In this case, we have a mixture of one sugar with low factor and one with high factor, but as these are mixed only mechanically and molasses on two sugars being separate, we cannot consider the average analysis as representing the factor of sugar which gave off highest molasses, as according to the theory, this sugar should be first to deteriorate.

"It would thus appear that for making good storage sugar, if we can depend on formula $\frac{H_2O}{100 - P}$*, that the best sugar would result where we use the two massecuite system in case sugar not over 96 is desired. By running purity of first massecuites as low as possible to get 96 test without washing we would then expect to get sugar with highest possible factor as shown by table.*

"If we use three massecuite system, we may get lower average moisture and high average polarized sugar, but according to formula keeping quality not as good."

"Also with the low moisture sugars and at same time low factor sugars, it will take less absorption of moisture to change factor to unsafe. For example we have a sugar 97 polarization, .96%

moisture with a factor .320, this being less than .333 we would call it safe. Suppose it now absorbs .3% moisture. It would then have pol. 96.71, moisture 1.26, giving factor .383, which would be considered unsafe."

Take now a sugar 96 pol. 1.12 moisture. Let this absorb .3% moisture; we have pol. 95.71, moisture 1.42, factor .331 which would still be safe."

"Now another consideration, which sugar will be the most hygroscopic? Will the lower polarization sugar absorb more moisture than the sugar of higher polarization? If such is true, the sugar having the better factor of safety might absorb additional water and become unsafe sooner than the sugar which gave off high purity mol., and has only a narrow margin of safety."

Evidently one of the surest means of manufacturing raw sugars which will not deteriorate in storage is to keep the density of the molasses films surrounding their crystals beyond 75 Brix. This applies, of course, only to raw sugars, as the keeping quality of white sugar must, in the nature of the case, depend upon their absolute dryness. But to manufacture sugars with a polarization of 96, with films of very low purity molasses, necessitates a thorough purging of the crystals so that they will retain only a small amount of their molasses; otherwise the polarization of the sugar would be too low. However, a good purging can only result from good and effective clarification, and it is to the deficiency in this respect that much of the trouble from deterioration is due. If the massecuite is viscous from excess of lime it does not purge well, and excessive amounts of water have to be used in the centrifugal. In regard to the theory of the relation between film density and sugar deterioration, Browne[1] points out that a case once came under his observation where a sugar house superintendent made it a practice to wash sugars in the centrifugal with low grade molasses instead of water. Although this practice resulted in the entire prevention of deterioration in storage, it is improbable that the superintendent fully understood the scientific principle upon which its success depended.

[1]Chemical Factor in the Deterioration of Raw Cane Sugars.

In connection with the consideration of the composition of sugars in its relation to deterioration, we should mention the influence of size of grain.

THE INFLUENCE OF SIZE AND HARDNESS OF GRAIN UPON SUGAR DETERIORATION.

It is an obvious fact that the smaller the grain of a sugar the greater will be the amount of film surface exposure. Hence, other conditions being equal, a small grain sugar is more apt to deteriorate than one with a larger grain, because it is harder to purge properly and offers more surface for moisture absorption.

Regarding the influence of hardness of the grain upon sugar deterioration but little seems to be known. It is generally believed, however, that the hardness of the grain plays a considerable part in protecting sugars against such changes. Hard grain sugar appears less hygroscopic than sugars of the soft grain type. However, the impression may have been obtained simply from the fact that hard grain sugars are usually dryer. Whether their hardness makes them easier to dry, or whether they are hard because of the thoroughness with which they have been dried, are questions which we have not attempted to answer. As regards the hygroscopic nature of sugars we will have more to say under a subsequent text.

THE INFLUENCE OF BAGACILLO UPON SUGAR DETERIORATION.

There seems to be a growing conviction in the minds of the Cuban sugar manufacturers that one of the principal causes of sugar deterioration is the presence of small particles of cane fibre (known as bagacillo in Spanish) in sugars. Browne[1] has shown that a comparative study of the amounts of this material occurring in good keeping and deteriorated samples of sugar tends to lend support to this claim. Thus he finds an average of .07% bagacillo in sugars which kept well, and an average of

[1]For a very thorough discussion of "The relations between grain size and surface film," the reader is referred to Otto Rahn's valuable work on "The Bacterial Activity in Soil as a Function of Grain Size and Moisture Content." Michigan Technical Bulletin No. 16.

0.16% in sugars which deteriorated. He does not, however, believe that it is a direct cause of the deterioration, but thinks it merely an indication of careless methods of manufacture, which in themselves are conducive towards deterioration.

The writer has made a number of experiments upon the influence of the addition of bagasse particles upon the deterioration of sugars. In every case where the conditions of the experiment precluded the influence of moisture retention by the bagasse the results were negative. In the writer's opinion bagacillo only influences sugar deterioration by the absorption and retention of moisture, by means of which a moist zone is created in which deterioration can take place. No importance need be attached to the theory of the bagacillo acting as a carrier of infection, for the most dangerous infection of sugars is introduced while it is in the centrifugal. The bagacillo, on the other hand, originates at the mill end of the factory process.

PROVIDING THE PROPER STORAGE CONDITIONS FOR SUGAR.

The prevention of sugar deterioration not only necessitates the manufacture of sugars whose molasses films conform in density to the factor of safety, but also upon the maintenance of this density. Applied to industrial conditions, this means that sugars must be stored in a relatively dry atmosphere. When we consider this question in its specific relation to Louisiana conditions we may disregard the question of the influence of certain impurities upon the hygroscopic nature of sugars. In this climate we need not consider the influence of glucose[1] or of certain ash constituents upon the moisture absorbing power of sugars. Where the humidity is so high as it is here, the operation of these influences is by no means necessary for the absorption of moisture by sugar. The writer has frequently observed grains of coarse standard granulated sugar almost completely deliquesce when exposed to the atmosphere on a very humid day. In this case we can hardly attribute the absorption of moisture to the hygroscopic properties of the sugar, but preferably to the pressure

[1]Deerr and Norris, The Deterioration of Sugar on Storage, Hawaiian Bulletin No. 24.

of atmospheric moisture and its resulting condensation upon solid particles. Under these conditions good warehousing is essential to the satisfactory keeping of sugars. Of the type of warehouse in which a dry atmosphere can be most effectively and most economically maintained not much needs to be said. Provision should be made for drying the warehouse when the humidity increases beyond a certain point. It is well to have concrete floors, but the sugar should not be piled directly thereon, but a false floor of boards with an air space between the two floors should be provided.

The changes in the composition of sugar due to the absorption of moisture are of great importance in connection with the sampling of cargoes for valuation according to polarization. The relative moisture absorbing powers of different types of sugar, under different conditions of atmospheric humidity, have been exhaustively studied by Browne.[1] The following table, taken from his "Handbook of Sugar Analysis," shows the gain or loss in the moisture of different sugars at 100% and at 60% relative humidities.

[1]Handbook of Sugar Analysis, p. 7.

TABLE II.

Kind of Sugar	Grain	Polarization	Moisture in Sugar	Gain first hour 100% humidity	Change first hour 60% humidity	Total change at point of equilibrium	Humidity at equilibrium	Residual moisture at equilibrium
			Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Granulated	Fine	99.85	0.10	1.78	+ 0.03	+ 0.01 (2 hrs.)	56	0.11
Peruvian	Large	98.40	0.35	1.09	− 0.09	− 0.14 (4 hrs.)	56	0.21
Porto Rican	Medium	96.40	1.31	1.40	− 0.54	− 0.73 (2 hrs.)	62	0.58
Philippine mats	Fine	87.45	3.12	1.80	− 0.68	− 1.25 (6 hrs.)	56	1.87
Cuban molasses	Large	82.75	4.85	1.12	− 1.00	− 2.42 (24 hrs.)	59	2.43

Handbook of Sugar Analysis pg. 7. C. A. Browne.

It will be noted in the above table that the samples of fine grain sugars absorbed more moisture than those of large grain. In discussing the absorption of moisture by sugars under excessive humidity, Browne states that no relationship can be traced in the above table between the composition of the sugar and rate of absorption. However, when the grains are compared it will be found that the smallest grain sugars have the largest absorptive powers. With regard to the evaporation of moisture from sugars, under diminished pressure, the author referred to states that here a very definite relationship exists between composition and rate of evaporation. The rate of evaporation being proportional to the initial moisture content, and the amount of moisture remaining in the sugar at the point of equilibrium, is a function of the hygroscopic quality of the nonsugars.

Prinsen Geerligs,[1] who has made an extensive investigation of the rate of moisture absorption by different sugars, reaches the following conclusion:

Refined sugars, and very high grade raw sugars, absorb moisture at a relative humidity of 80 or over, when the temperature is between 24 and 33°C. The limit for white first sugar is 75, 70 for refinery crystals and molasses sugar, and between 70 and 75 for seconds, according to quality.

In the following table the average mean temperature, in degrees centigrade, is given for nine months of the year in Louisiana, also the average relative humidities for the respective months. The humidities from January to August are for the year 1917, which was unusually dry from March until July. The humidities given for these months are therefore lower than in normal years.

[1]Cane Sugar and its manufacture, pp. 298, 299.

TABLE III.

Table Showing Average Mean Temperature (Centigrade Degrees) and Average per cent Humidity for the Months of the Grinding Season in Louisiana, and the seven months following thereafter.

Month	Mean Temperature	% Humidity
October	20.8	79
November	16.6	68
December	14.5	81
January	15.6	81
February	15.2	78
March	19.0	72
April	19.70	68
May	21.80	63
June	26.00	65
July	27.72	74

From the table it will be seen that conditions of temperature and humidity in Louisiana make adequate warehousing essential to the prevention of sugar deterioration in storage.

THE COLD STORAGE OF SUGARS.

It has sometimes been suggested in connection with the question of warehousing sugars, that the principle of cold storage should be combined with that of dry storage. This, in fact, has been seriously considered in connection with the warehousing of the plantation granulated sugar output of one of the large white sugarhouses in this State.

In the following table are the results of a series of experiments upon the relative deterioration of sugars stored in an incubator in a refrigerator and at room temperature respectively.

TABLE IV.

Experiment upon Influence of Temperature upon Sugar Deterioration. Period of Incubation, one month.

Sample	% Moist.	Sucrose		% R. S.	Dry Basis		Factor	Temp.
		S. P.	S. C.		S. P.	S. C.		
Incubator	3.72	91.15	92.36	2.63	94.67	95.92	.420	34°C
Room Temp.	3.30	90.60	91.81	2.94	93.69	94.94	.351	—
Refrigerator	3.45	94.35	94.85	0.352	97.72	98.23	.610	Mean 20°C
Incubator	1.90	94.95	95.65	—	96.77	97.40	.376	—
Room Temp.	0.50	95.60	96.46	—	96.00	96.90	.113	—
Refrigerator	1.42	95.70	96.54	—	97.78	97.93	.327	—
Room Temp.	2.44	92.90	93.85	2.63	95.22	96.09	.343	—
Refrigerator	2.60	95.60	96.27	1.00	98.15	98.83	.590	—

It will be noted that all of the samples contained sufficient moisture to cause them to deteriorate. However, in every case the refrigerator stored sample deteriorated very slightly as compared to the others. This could only be attributed to the temperature at which the respective samples were stored. The mean temperature of the refrigerator was about 20°C., while the temperature of the incubator was 34°C. The experiment was carried out during the summer months, so that the room temperature was in some cases more favorable for the development of micro-organisms than that of the incubator.

PRECAUTIONARY MEASURES FOR THE PREVENTION OF THE INFECTION OF SUGARS WITH DETERIORATIVE MICRO-ORGANISMS.

While no very conclusive data has as yet been obtained upon the relations between the degree of infection of sugars and their rate of deterioration in storage, yet more and more stress seems to be laid upon the importance of cleanliness in the factory as a preventive measure. Not only so, but wherever the maxim of cleanliness is applied, gratifying results appear to follow. In Hawaii, losses from sugar deterioration seem to have been about entirely eliminated, and there the correction of this trouble is generally believed to have been effected by improving the cleanliness in the factory.[1] Although the writer has been one of the greatest advocates of cleanliness in the factory as a preventive measure against sugar deterioration, he has found it difficult in the light of previous investigation to explain the beneficial effects of this practice. So long as spore-forming bacteria were credited with playing the principal role in the deterioration of sugars, it was difficult to understand how cleanliness could eliminate or even reduce the infection of the products. Since the spores of these bacteria could successfully withstand all of the heating processes incident to manufacture, their elimination was a matter of impossibility, and a material reduction in the initial number of them in the finished sugar could only result from an almost

[1]See the report of Hawaiian Sugar Planters' Association, 1913.

perfect clarification. How, then, could cleanliness eliminate the cuasative agencies in sugar deterioration?

In connection with the consideration of the question of the importance of preventing the infection of sugar with micro-organisms, it is interesting to note the recommendation of Kamerling's[1] that all bags and material used in the packing of sugars should be disinfected. For this purpose he recommended the use of a hot one per cent solution of carbolic acid. The mats and bags were dipped into this solution and dried. Results reported in Prinsen Geerlig's Cane Sugar and Its Manufacture, from experiments on the comparative rate of deterioration of disinfected and non-disinfected samples, show that no benefit was derived from this method of treating the bags. These results were to have been expected since in all probability freshly made sugars are much more likely to carry such infection than is packing material.

To determine the effect of heating upon the deterioration of sugars in storage, the following experiment was conducted. A 96-test sugar was placed under moist chambers and allowed to become very moist. The sugar was then divided into twelve equal portions, of 200 grams, and put in Erlenmeyer flasks. The flasks were inoculated with several cultures of mould, then plugged with cotton, and heated to the temperatures shown in the table.

[1]Cane Sugar and its Manufacture. Prinsen Geerligs, p. 292.

TABLE V.

Experiment Showing Influences of Heat on Keeping Quality of Sugar.
One month Incubation.

Treatment	% Moist.	Sucrose		Sucrose Dry Basis		% R. S.	Factor
		S. P.	S. C.	S. P.	S. C.		
Control	4.00	90.3	91.23	93.98	94.91	2.45	.412
I 5′ @ 60°	3.90	90.7	91.71	94.38	95.39	2.69	.419
II 10′ @ 60°	3.79	90.65	91.75	95.36	96.46	2.77	.405
III 5′ @ 65°	3.89	90.2	91.39	93.85	95.04	2.39	.396
IV 10′ @ 65°	3.71	90.7	91.79	94.19	95.28	2.43	.398
V 5′ @ 70°	3.87	90.5	91.47	94.14	95.11	2.60	.407
VI 10′ @ 70°	3.82	90.4	91.23	93.99	94.82	2.26	.397
VII 5′ @ 75°	3.03	91.0	91.86	93.84	94.70	2.16	.336
VIII 10′ @ 75°	3.73	91.1	91.94	94.72	95.56	2.46	.419
IX 5′ @ 80°	3.80	91.4	92.26	95.01	95.87	2.13	.441
X 5′ @ 85°	3.74	91.7	92.26	95.26	95.82	2.10	.450
XI 5′ @ 90°	4.36	91.7	92.34	95.88	96.52	1.81	.525

Original Analysis —
Dry Basis — S. P. = 96.89
S. C. = 97.23

The results of the above table show that heating the sugar for five minutes at 90°C. decreased its deterioration greatly, as compared with the unheated control. Also at 80° and 85° for five minutes the deterioration was much less than in the control flask.

More recent investigations, the results of which have been reported in a previous part of this bulletin, show that bacteria play an almost insignificant role in the average case of sugar deterioration. Moulds are now known to be the most dangerous of all the micro-organisms occurring in sugar. Furthermore, they have a comparatively low thermal death point. As an example, Thom[1] has shown that in the flash process of pasteurization, where milk is heated to 165°F. (73.9°C.) for a period of 30 seconds the spores of most of the mould species were killed. At

175°F. (79.5°C.) only occasional spores developed. When the heating was performed in dry air at a temperature of 30 seconds, at 200°F. (93.3°C.) seven out of twenty-four forms of aspergillus were destroyed.

In the light of these results, the elimination of moulds from sugars seems entirely feasible. On the contrary, it can be well understood that a heavy initial infection of sugars with these spores indicates a condition of uncleanliness existing around the centrifugal which could be easily and profitably corrected. In connection with these facts, Shorey's[1] recommendation for covering the centrifugals, and for using steam for washing sugars, seems to offer a thoroughly practical method for the elimination of one of the most dangerous group of micro-organisms associated with sugar deterioration.

The possibility of decreasing the deterioration of sugars, by pasteurization, is not merely a matter of inference. From the results shown in the above table the writer credits to the effect of such pasteurization the large number of negative results obtained from sugars which were heated to 90°C. for one hour before being inoculated with bacteria. At that time it was not so fully known that bacteria can only cause the deterioration of very moist sugars, so the negative results were obtained in most cases from samples having a factor of .400 to .45.

CONCLUSIONS.

(1) Sugar deterioration is caused by moulds, torula, and in some cases by bacteria.

(2) The mould group includes aspergillus fumigatus, a blue aspergillus, and floccose strains and aspergillus pencilloides.

(3) The moulds constitute the most dangerous group of micro-organisms in sugars, on account of their strong inverting power, their ability to exercise this power in highly concentrated sucrose solutions, and in solutions of varying reaction; also on account of their ability to develop on medium very deficient in nutrients.

(4) The torula are of constant occurrence in sugars. While they are active in destroying reducing sugars, under widely

[1]Effect of Pasteurization upon mould spores. Journal of Agricultureal Research, Vol. 6 No. 4.

varying conditions, their ability to invert sucrose is comparatively weak, and only exercised under especially favorable conditions.

(5) The bacteria can only cause the deterioration of sugars which have absorbed a comparatively large amount of moisture, and which have as a consequence a very low solid non sucrose moisture ratio.

(6) The "Factor of Safety" of the Colonial Sugars Company of Australia is a remarkably good criterion of the keeping quality of sugars. Its validity is believed to be due to the fact that it indicates a certain density of the molasses films surrounding the crystals of the sugar. It is of less value in predicting the behavior in storage of washed 96-test sugars. It is inapplicable to white sugars, because in such cases, it is the total moisture that must be considered since the density of the film cannot exceed the maximum concentration in which micro-organisms can destroy sucrose.

(7) Cleanliness in the sugar factory, and particularly around the centrifugals, offers a partial means for eliminating the infection of sugars. The use of covered centrifugals, and of steam as a substitute for wash water is to be recommended.

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